The Journal of Parasitology

Volume 9 DECEMBER, 1922 Number 2

THE EFFECTS OF CHANGES IN DIET ON THE INCIDENCE, DISTRIBUTION AND NUMBERS OF CERTAIN INTESTINAL PROTOZOA OF FROG AND TOAD TADPOLES *

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So far as known to the writer no studies have been published on the relations between the diet of an animal and the incidence, distribution and numbers of the intestinal protozoa with which it may be infected. In the following pages are recorded the results of some investigations in this field. The host animals used were principally tadpoles of the green frog, *Rana clamitans,* and of the leopard frog, *R. pipiens,* and toad, *Bufo lentiginosus americanus.* The protozoa studied were two ciliates of the genera Opalina and Nyctotherus, and two flagellates of the genera Trichomonas and Hexamitus. Other protozoa are more or less regular inhabitants of the digestive tract of these tadpoles but the four mentioned are usually numerous and easily found and are often present in 100 per cent. of the tadpoles examined. Tadpoles of the green frog were selected for special study for several reasons: (1) they do not metamorphose the first season and hence can be obtained, at least in the southern part of their range, at any time of the year; (2) they are hardy and can be subjected to severe experimental conditions without great mortality; (3) they are usually well infected with intestinal protozoa, and (4) one type of protozoon, an Opalina, is an inhabitant of the tadpole but not of the adult (Metcalf, in litt.),† a condition that affords an interesting problem for investigation. In certain other species of frogs and toads the infection with Opalina appears to be continuous from tadpole to adult.

The observations and experiments described below involve a study of (1) changes that occur in the protozoan fauna of the green frog during the tadpole stage and the process of metamorphosis; (2) factors

* This is the first of a series of papers on the relation between diet and intestinal protozoa from the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University. The writer is indebted to Dr. H. D. Reed for many courtesies during the summer of 1922 while working in the Zoological Laboratory at Cornell University.

† Kudo has recently reported an opalina from this species but this apparently is of rare occurrence.

that bring about the loss of Opalina in the adult frog, and (3) the failure of the adult green frog to become reinfected. The incidence of infection, distribution within the alimentary canal, and numbers of intestinal protozoa present were obtained for several stages in the growth and metamorphosis of the normal tadpole; some experiments on the effects of starvation on the protozoan fauna were carried out; and the results of various changes in diet were determined.

I. *Observations on the Course of Infection with Intestinal Protozoa —During the Growth and Metamorphosis of Frog and Toad Tadpoles.*— As stated above, Opalina, with one exception, has not been recorded from adults of the green frog, *Rana clamitans.* The course of infection with this genus of protozoon during the growth and metamorphosis of the larvae of this species has not been reported. It is of interest in connection with the experimental work described below to determine at what time the tadpoles become infected and when and why they lose their infection. The writer examined a number of larvae of the green frog that were collected on July 25, 1922, about ten days after hatching. Every one of them was infected with Opalina—some of them abundantly infected with this form—as well as with Nyctotherus, Trichomonas and Hexamitus. The tadpoles of *R. clamitans* are not gregarious but scatter widely soon after hatching, hence infection by close association with one another seems improbable. The high rate of infection and large numbers present at the early date of examination, therefore, indicates a strong susceptibility to infection of the tadpole. The larval life of the green frog extends over two seasons, and in the vicinity of Baltimore it is possible to obtain tadpoles during the winter months. Examinations of many specimens collected at various times of the year has shown that Opalina as well as the other three genera named above are inhabitans of the rectum of *R. clamitans* throughout larval life.

In 1922 an opportunity was afforded to study the intestinal protozoa of metamorphosing tadpoles. The most noticeable internal changes during metamorphosis are the shortening and differentiation of the intestine and rectum. During these changes the number and distribution of the Opalinae were affected as indicated in Table 1 and Figure 1. Here are presented data from a study of 15 specimens arranged in 3 groups on the basis of length of intestine and presence and length of fore and hind legs. The length of the rectum is difficult to determine because it is tightly coiled, hence the measurements given for this section are only approximate.

1. The five specimens in an "early" stage of metamorphosis were without fore legs and possessed intestines ranging from 320 to 265 mm. in length. In the rectum of these Opalinae were present in large

numbers—designated by "many" or "abundant." No Opalinae were found in the intestines except in one case in which there were many.

2. A second group of five tadpoles are termed "intermediates." The intestines of these were 245 to 138 mm. in length and there were no forelegs. Opalinae were numerous in the rectum of two, rare in two and absent in one; they were present in very small numbers in the intestines of all five tadpoles.

3. The third group of five were in a "late" stage of metamorphosis. Their intestines ranged from 145 to 38 mm. in length and in all of them the forelegs had emerged. No opalinas were found in the rectum of any of them and in the intestine of only one. This specimen had a comparatively long intestine and only a few ciliates were present.

TABLE 1.—Measurements of Metamorphosing Tadpoles of the Green Frog in Early, Intermediate and Late Stages Showing the Relation Between Length of Intestine and Rectum and Number of Opalinae Present

Stage of Metamorphosis	Length in Mm. of				Length in Mm. of		Number of Opalinae in	
	Body	Tail	Fore Legs	Hind Legs	Intestine	Rectum	Intestine	Rectum
Early	24	48	0	20	320	45	Many	Many
Early	29	63	0	28	298	35	0	Many
Early	28	66	0	30	272	40	0	Abundant
Early	25	45	0	12	270	25	0	Abundant
Early	27	45	0	22	265	45	0	Many
Intermediate	29	60	0	36	245	30	Few	Many
Intermediate	27	53	0	24	220	25	Few	0
Intermediate	27	61	0	27	215	35	Rare	Rare
Intermediate	28	55	0	39	170	25	Few	Rare
Intermediate	28	64	0	33	138	25	Rare	Many
Late	28	55	13	40	145	22	Few	0
Late	30	64	15	37	130	20	0	0
Late	28	59	17	41	70	10	0	0
Late	30	57	18	50	44	7	0	0
Late	27	50	20	47	38	7	0	0

The other intestinal protozoa differed markedly from Opalina in numbers and distribution. Nyctotherus was present in the rectum of all of the 15 tadpoles examined, being numerous in all of the early stages, in two of the intermediates and three of the late stages. No specimens of this form were found in the intestines of the early stages, a few were present in one of the intermediates and several or a few in every one of the late stages. Trichomonas occurred in the rectum of all of the early and intermediate stages but in only one of the late stages. It was not numerous in any of them. With the exception of one intermediate, this form was absent from the intestines of all of the 15 examined. Hexamitus was found to be abundant or moderate in numbers in the rectum of all of the tadpoles but only a few were present in the intestines of 6 of them.

Data from a larger number of tadpoles would make conclusions more definite, but the findings indicate that Opalinae are normally

present in considerable numbers in the rectum of tadpoles of the green frog from a few days after hatching until the time of metamorphosis. During the period when the intestine decreases in length from about 300 to 150 mm. the Opalinae decrease in numbers or disappear entirely from the rectum and some of them appear to migrate into the intestine. After the forelegs emerge, Opalinae are entirely absent from the rectum and from the intestine of most of the tadpoles. No significant differences, on the other hand, were noted in the numbers and distribution of Nyctotherus, Trichomonas and Hexamitus during metamorphosis.

The number and distribution of Opalinae during the metamorphosis of tadpoles of *Rana pipiens* and of the toad were also obtained in a few cases. These data are of interest for comparison with those noted above because, although adults of both of these species harbor Opalinae they might lose their infection during metamorphosis, as in the green frog, and become reinfected after they are fully transformed. Three metamorphosing tadpoles of *R. pipiens* were examined on July 5 and 3 fully transformed specimens on July 25, 1922. Opalinae were present in the rectum of 5 of the 6 specimens, being absent in 1 of the young frogs; they were also found in the intestine of 4 of the specimens, being absent from 1 of the metamorphosing tadpoles and from 1 of the fully transformed frogs. It seems probable, therefore, that in this species Opalinae persist throughout the period of metamorphosis and that the infection of the adult is a continuation of that of the tadpole. Data obtained from the examination of toad tadpoles indicate that this is true also in this species. Ten toad tadpoles collected on June 12, 1922, were examined on June 28, at which time their average measurements were as follows: body, 8 mm.; tail, 12 mm.; hind legs, 3 mm.; intestine, 47 mm.; rectum, 9 mm. Five of these 10 specimens contained Opalinae. The absence of Opalinae in the other 5 was probably due to loss due to confinement without food for 16 days (see page 55 on effects of starvation on the protozoan fauna of the intestine).

The results of the observations on metamorphosing green frog tadpoles, as regards Opalinae, are shown graphically in Figure 1. In the early stage of metamorphosis the Opalinae are numerous in the rectum but seldom present in the intestine. In the intermediate stages the number in the intestine and rectum is about the same and the total number in the 2 sections probably equals that in the early stages. What apparently has happened is that ciliates have migrated from the rectum into the intestine. In the late stages the Opalinae disappear from the rectum first and then from the intestine.

II. *The Effects of Starvation of the Host on Intestinal Protozoa.*—The factors of the environment of intestinal protozoa differ considerably from those of free-living species. In such an animal as the tadpole

the light that penetrates the intestinal wall must be very feeble; the medium within the intestine is comparatively viscous; the possibility of being swept away as the food masses pass through must be guarded against; the chemical conditions are variable and complex due to digestive juices, the products of digestion, and other biochemical products produced by the host; and locomotion is difficult and requires more energy than in free-living species. Among the important environmental factors is, of course, the food supply. One cannot tell as yet the exact nature of the food of any of the protozoa dealt with in this paper, but there certainly must be a marked change in the intestinal contents of the tadpole under starvation conditions which might affect favorably or unfavorably the protozoan fauna. Tadpoles of the toad and of *Rana*

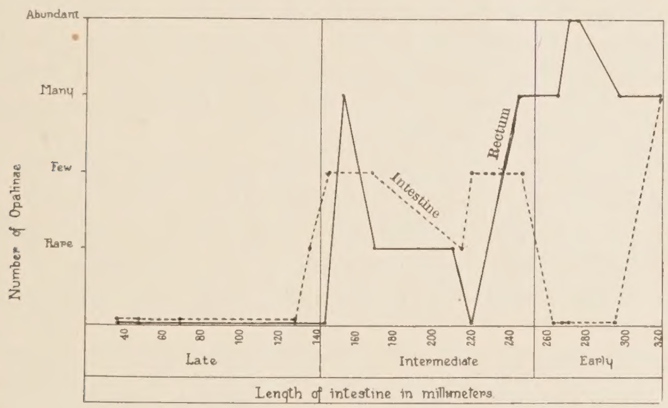

Fig. 1.—Curves showing the comparative numbers of Opalinae present in the intestine (broken line) and rectum (solid line) of 15 tadpoles of the green frog in early, intermediate and late stages of metamorphosis.

pipiens and *R. clamitans* were kept for various periods in the laboratory in glass dishes without food, the water being changed frequently thus preventing the continued reingestion of materials that had already passed through the digestive tract. Opalina, Nyctotherus, Trichomonas, and Hexamitus were present in almost all tadpoles at the time they were collected from the ponds. Examinations of the rectum and intestine gave, in brief, the following results:

1. Opalinae were found in *R. pipiens* up to the 9th day in 3 specimens but was absent in four specimens examined on the 10th, 12th, 22d and 25th days, respectively. They were present in small numbers in 4 specimens of *R. clamitans* examined on the 12th day, although abundant in specimens direct from the ponds. Fourteen toad tadpoles dissected from the 16th to the 37th day revealed Opalinae in small numbers in only 2 specimens on the 16th day. Thus lack of food under laboratory conditions appears to be decidedly detrimental to Opalina.

2. Nyctotherus seems to disappear more rapidly than Opalina. None were found in 7 specimens of *R. pipiens* after from 7 to 25 days, nor in 4 specimens of *R. clamitans* after 12 days, and in only 2 of 14 specimens of toad tadpoles after 16 days.

3. Trichomonas was not very abundant in any of the tadpoles examined and data regarding their persistence or disappearance are not definite.

4. Hexamitus did not seem to suffer particularly in the toad tadpoles, being present in large numbers after 29 and 37 days, respectively, although there was a falling off in numbers in tadpoles of *R. pipiens* in 22 and 25 days and in *R. clamitans* in 12 days. It may be concluded from these data that starvation under laboratory conditions is more or less disadvantageous to the species of protozoa studied. Previous observations on the intestinal fauna of tadpoles has given me the general impression that these protozoa do not disappear at all or at least not so rapidly under laboratory conditions when their hosts are well fed, but I have no definite data to prove this.

III. *The effects of Changes in the Diet of the Host on the Incidence, Distribution and Numbers of Intestinal Protozoa.*—In selecting food substances that may have an influence on the intestinal protozoa of tadpoles, materials from the ductless glands are immediately suggested, because of the remarkable modifications they have been shown to bring about.

Since Gudernatsch (1912) first reported the effects of a diet containing thyroid substance on the metamorphosis of frog tadpoles, many investigators have subjected protozoa to this and to materials from other ductless glands as well as to substances in which vitamines of different types were either present or absent. Many of the efforts in this direction were negative or indefinite but others have been positive. Shumway (1917), for example, has shown conclusively that the division rate of Paramecium is increased about 65 per cent. by a diet of dessicated thyroid. The hormone in thyroid has been found to act as a chemical stimulus to other tissues causing increased metabolism. In the case of Paramecium this increased metabolism probably results in more rapid growth and hence a higher rate of division.

So far as I know none of the thousands of tadpoles used for experiments on the feeding of glandular substances or on the effects of removing or grafting ductless glands have been examined to see whether any changes had occurred in the intestinal fauna. The questions involved in the investigations described below are: 1. Do glandular and other substances eaten by the host affect the rate of division of intestinal protozoa as they do of free living species? 2. Is the incidence of infection and distribution within the digestive tract changed by

radical changes in the diet of the host? 3. Can the incidence, numbers and distribution of the protozoa be correlated with changes in the host due to modifications of the diet?

The tadpoles of the green frog, *Rana clamitans,* were used as experimental animals and the protozoa studied were principally Opalina, Nyctotherus, Trichomonas, and Hexamitus. The green frog, as mentioned above, is of particular interest since the large ciliate, Opalina, occurs in large numbers in the tadpoles, but is almost never found in the adults. The principal material used as food was dessicated thyroid substance. This was selected because it has been shown to have a marked influence on the division rate of certain free-living protozoa and on the metamorphosis of tadpoles. The other glandular substances used were thymus, ovary, orchic, prostate, pituitary, and suprarenal.

1. *Preliminary Experiments.*—Several preliminary experiments were carried out in January and March, 1921, for the purpose of perfecting methods and of determining how many tadpoles were necessary to give significant results. One group of 6 tadpoles furnished material for an experiment lasting 9 days (January 19 to 28), and another group of 15 for an experiment lasting 7 days (March 7 to 14). It was found that tadpoles of the green frog when fed on desiccated thyroids mixed with flour began to die on the 7th day and that considerable mortality occurred on the 8th and 9th days. Evidences of external metamorphosis were conspicuous; the body changed considerably in shape and decreased in length about 20 per cent., and hind legs were developed. The most interesting internal change noted was a decrease in the length of the intestine of about 50 per cent. No significant data were obtained regarding the intestinal protozoa.

2. *Further Experiments in 1921 with Desiccated and Powdered Thyroid Glands, Thymus Glands, Ovaries, and Orchic Substance.*—In these experiments the controls were fed on flour that had been made into a paste with water, spread on glass plates to dry, and then crumbled (Swingle, 1918). The experimental animals were fed on 7 parts flour plus 3 parts desiccated and powdered glandular substance prepared in the same way. The tadpoles were kept in circular glass dishes 22 cm. in diameter. The water and food were changed daily, 1 liter of tap water being provided for each dish and an excess of food added. The data obtained are given in Table 2. No very definite method was devised of determining the exact number of protozoa present. The entire rectum of each tadpole was stirred up on a slide with about 0.1 c.c. of normal saline solution and part of this covered with an 18 mm. square cover glass. A binocular dissecting microscope was used to detect the presence of ciliates and a compound microscope with

number 5 ocular and 16 mm. and 4 mm. objectives was then employed to determine the numbers of both ciliates and flagellates.

The tadpoles proved to be favorable material for experimental purposes since very few of them died and the incidence of infection was high; 80 per cent. were infected with Nyctotherus, 90 per cent. with Opalina, 85 per cent. with Trichomonas, and 95 per cent. with Hexamitus. A comparison of the controls and experimental tadpoles is possible with the data contained in Table 2.

(*a*) *Thyroid Experiments.*—Tadpoles fed on desiccated thyroids and examined at the end of 5 days changed both externally and internally as in the preliminary experiments. The rectum contained less material than that of the controls and both the incidence of infection

TABLE 2.—Incidence of Infection and Comparative Numbers Present of Two Ciliates, Nyctotherus and Opalina, and Two Flagellates, Trichomonas and Hexamitus, in the Rectum of Tadpoles of the Green Frog, *Rana clamitans*, and the Bull Frog, *R. catesbiana*, After Being Fed on Various Glandular Substances

		Nyctotherus			Opalina			Trichomonas			Hexamitus		
Glandular Substance	No. of Tadpoles	Per Cent. Infected	Per Cent. with Many	Per Cent. with Few	Per Cent. Infected	Per Cent. with Many	Per Cent. with Few	Per Cent. Infected	Per Cent. with Many	Per Cent. with Few	Per Cent. Infected	Per Cent. with Many	Per Cent. with Few
Control..	20	80	15	65	90	65	25	85	15	70	95	90	5
Thyroid..	27	26	3.7	22	4	0	4	11	0	11	89	52	37
Thymus..	17	76	41.2	35	35	11.7	24	59	12	47	82	59	24
Ovary....	20	75	25	50	45	15	30	85	35	50	85	55	30
Orchic....	17	71	46.9	24	29	5.8	24	71	29	41	29	6	24

and number present decreased for all four protozoa recorded. The incidence of infection with Nyctotherus decreased 66 per cent. and the numbers when present decreased about 50 per cent. This result is very different from that obtained when a similar free-living ciliate, Paramecium, is fed on thyroid substance, division rate in the latter being accelerated about 65 per cent. (Shumway, 1917). There seems to be no reason why Nyctotherus should not react to thyroid feeding by dividing more rapidly the way Paramecium does. The difference noted may be due (1) to a change in the thyroid substance before it reaches the rectum such as the absorption of the effective hormone, or (2) to the change in the intestinal tract characteristic of thyroid-fed tadpoles. Opalina and Trichomonas were almost eliminated from the thyroid-fed tadpoles and Hexamitus, although present in almost as many specimens, was not so numerous.

(*b*) *Thymus Experiments.*—Seventeen tadpoles were examined after six days of feeding on dessicated thymus. Nyctotherus decreased silghtly in incidence but increased considerably in numbers. Opalina decreased about 60 per cent. in incidence and also in numbers. The incidence of Trichomonas fell off about 30 per cent., but an increase

in numbers took place. Hexamitus decreased slightly in incidence and considerably in numbers.

(*c*) *Ovary Experiments.*—In the 20 tadpoles fed on desiccated ovarian substance for six days, Nyctotherus decreased slightly in incidence but increased in numbers; Opalina decreased 50 per cent. in incidence and also in numbers; Trichomonas increased in numbers, and Hexamitus decreased slightly in both incidence and numbers.

(*d*) *Orchic Experiments.*—The 17 tadpoles fed on desiccated orchic substance showed a slight decrease in the incidence of Nyctotherus but an increase in numbers; a 66 per cent. decrease in the incidence of Opalina and a corresponding decrease in numbers; a slight decrease in the incidence of Trichomonas accompanied by an increase in numbers; and about a 70 per cent. decrease in incidence of Hexamitus with a great decrease in numbers.

If one considers the four organisms under consideration separately on finds Nyctotherus adversely affected by thyroid feeding, and slightly reduced in incidence but increased in numbers, when present, by thymus, ovary, and orchic substance. The incidence of Opalina and numbers present decreased considerably in all the experimental tadpoles. Thyroid and thymus feeding reduced radically the incidence of Trichomonas but thymus, ovary and orchic substance caused an increase in numbers when present. The incidence of Hexamitus was not greatly influenced except by orchic substance which caused a great decrease. The number of Hexamitus present decreased in all of the experiments.

The organisms selected for study comprise two species that are holozoic, Nyctotherus and Trichomonas, and two, whose method of nutrition is osmotic, Opalina and Hexamitus. The data obtained show that the former suffered less than the latter during the course of the experiments. In tadpoles fed on thymus, ovary, and orchic substance the incidence of infection with Nyctotherus was almost as high as in the controls and there was an actual increase in the numbers present. The data show similar results with Trichomonas although in thymus-fed tadpoles the incidence fell about 33 per cent. On the other hand, the incidence of Opalina in tadpoles fed on these 3 substances fell from 50 to 67 per cent. and a decrease in numbers was observed; and in Hexamitus, although the incidence decreased markedly only in orchic-fed animals, the numbers likewise decreased considerably.

The number of tadpoles studied was small and small variation in incidence and number are therefore not significant, but it seems clear that the 2 protozoa that ingest solid particles, Nyctotherus and Trichomonas, were, on the whole, not unfavorably affected by the diets used and may even have been stimulated to more rapid growth and division. The other 2 protozoa with the osmotic type of nutrition, Opalina

and Hexamitus, seem to have been acted upon unfavorably, due probably to the absorption of deleterious substances in solution.

3. *Experiments in 1922 with Thyroid Glands, Prostate Glands, Pituitary Glands, Suprarenals, Orchic Substance, and Meat.*—Sixty one-year-old tadpoles of the green frog and bull frog were used for each diet, 20 being placed in each dish and fed as described above. The results of these extensive feeding experiments are not as satisfactory as expected because the incidence of infection with Nyctotherus and Opalina was very low, namely, 15 per cent. with the former and 5 per cent. with the latter. Data regarding these two species have been omitted from Table 3 on this account since the results were not significant. For obtaining a basis for the comparison of the numbers of protozoa present, the number of each species was counted in 10 fields of the rectal contents of each tadpole using a 4 mm. objective and a No. 10 ocular and an average taken. The data show that Trichomonas

TABLE 3.—Incidence of Infection and Comparative Numbers Present of Two Flagellates, Trichomonas and Hexamitus, in the Rectum of Tadpoles of the Green Frog and Bull Frog, After Being Fed on Various Substances

		Trichomonas		Hexamitus	
Substance	Number of Tadpoles	Per Cent. Infected	Average Number per Field	Per Cent. Infected	Average Number per Field
Control	39	92	6	90	9.3
Thyroid	50	16	0.27	76	3.2
Prostate	19	50	0.2	85	9.0
Pituitary	20	90	5	100	35.2
Suprarenal	10	80	2.34	90	15.5
Orchic	10	100	6.5	100	34.2
Meat	25	75	4.1	100	16.7

suffered severely in the thyroid-fed tadpoles, both in incidence of infection and in numbers present. Decreases almost as great occurred in prostate-fed tadpoles. The intestine of tadpoles fed on these substances are profoundly affected, undergoing rapid metamorphosis (Swingle, 1918; Hegner, 1922) a phenomenon that may account for these decreases rather than the direct action of the ingested substances. In no case does the experimental diet seem to have had a particularly favorable effect on Trichomonas. Hexamitus also decreased in the thyroid-fed tadpoles but not to such an extent as did Trichomonas. The other diets seem to have favored its growth and multiplication, especially pituitary and orchic substance. This result does not agree with the experiments of 1921 (Table 2) in which a marked decrease in incidence and numbers of Hexamitus was noted in tadpoles fed on orchic substance.

4. *Further Experiments in 1922 with Thyroid Substance.*—During the month of June, 1922, opportunity was afforded to carry on another series of experiments with thyroid substance at Cornell University,

Ithaca, N. Y. One-year-old green frog tadpoles were collected on June 14 and fed on thyroid substance daily until June 18 or 19. Controls collected on the same date were examined on June 17. During the 4 or 5 days of the experiment evidences of both external and internal metamorphosis appeared. Table 4 gives measurements of 10 specimens each of normal and thyroid-fed tadpoles. The most noticeable changes are an average decrease of about 20 per cent. in the length of the body and of about 40 per cent. in the length of the tail of the thyroid-fed specimens and an average increase in the length of the hind legs of these same specimens of about 93 per cent.

The only internal changes studied were those involving the digestive tract. The rectum was difficult to measure on account of its coils and hence the data with regard to this section of the alimentary canal are

TABLE 4.—Measurements in Mm. of Ten Specimens Each of Control Tadpoles of the Green Frog, and Tadpoles That Had Been Fed on Thyroid Substance for Five Days

	Nyctotherus Length of Body		Opalina Length of Tail		Trichomonas Length of Hind Legs		Hexamitus Total Length	
	Range	Average	Range	Average	Range	Average	Range	Average
Controls	20–26	21.4	27–46	32.8	2–3.5	2.75	47–72	54.2
Thyroid-fed	15–19	17.2	13–29	19.8	3–8	5.3	29–48	37

TABLE 5.—Measurements in Mm. of the Intestine and Rectum of Twenty-Five Specimens Each of Control Tadpoles of the Green Frog and Tadpoles That Had Been Fed on Thyroid Substance for Four or Five Days

	Length of Intestine		Length of Rectum	
	Range	Average	Range	Average
Normal	180–309	214.6	18–40	25.2
Thyroid-fed	82–188	112.2	10–25	14

only approximate. The differences in the measurements of the intestine and rectum of twenty-five specimens each of control and thyroid-fed tadpoles are shown in Table 5. The intestine and rectum of the thyroid-fed tadpoles decreased about 50 per cent. in length during the 4 or 5 days they were fed on this diet.

Number and distribution of Nyctotherus, Opalina, Trichomonas, and Hexamitus. As in previous experiments the entire contents of the rectum of each specimen was thoroughly mixed with a measured amount of normal saline solution; in these experiments 0.1 c.c. was used. Counts were made as follows. The diluted rectal contents were spread over approximately one-half of a 1 by three inch slide; an 18 mm. square cover slip was then placed on part of it; the average number of Opalinae present in ten microscopic fields using a 16 mm. objective and a No. 10 ocular was obtained, and of Trichomonas and Hexamitus

in ten microscopic fields using a 4 mm. objective and No. 10 ocular. The results are shown in Table 6 and in Figures 2 and 3.

According to the data in Table 6 the feeding of thyroid substance to green frog tadpoles has a decidedly unfavorable effect on the protozoan inhabitants of the rectum. Nyctotherus and Trichomonas were almost eliminated; and although the incidence of infection with Opalina and Hexamitus decreased only slightly, there was a very striking decrease in the numbers of these species when present. These results correspond fairly well with those obtained in the previous experiments described above. No better tadpoles for experimental purposes could be obtained than those used in this series since there was 100 per cent. of infection with all of the protozoa studied. Figure 2 shows by means of curves the relations between the length of the

TABLE 6.—Comparative Numbers of Nyctotherus, Opalina, Trichomonas, and Hexamitus in the Rectum of Twenty-Five Specimens Each of Control Tadpoles of the Green Frog and of Tadpoles That Had Been Fed on Thyroid Substance for Four or Five Days

(For method of obtaining these numbers see text)

	Per Cent. Infected	Number per Field		Per Cent. Infected	Number per Field		Per Cent. Infected	Number per Field		Per Cent. Infected	Number per Field	
		Range	Average		Range	Average		Range	Average		Range	Average
Normal.......	100	Present (1)	Present (1)	100	1–30	7.16	100	3–16	7.28	100	2–20	8.48
Thyroid-fed...	8	Present (1)	Present (1)	84	0.1–3	0.44	20	0–8 (2)	Few (2)	92	0–6 (3)	Few (3)

1. Nyctotherus was present in every specimen but too few in numbers to give a satisfactory count.

2. Trichomonas was present in moderate numbers in only three specimens, and rare in two other specimens.

3. Hexamitus was present in moderate numbers in 12 specimens but rare or few in number in 11 other specimens.

rectum and number of Opalinae present in the rectum of the twenty-five control and twenty-five thyroid-fed tadpoles, and brings out clearly the fact that a diet of thyroid causes a marked decrease in the length of the rectum and a corresponding decrease in the number of Opalinae present.

Protozoa in the Intestine.—The intestine of the 25 control and 25 thyroid-fed tadpoles were also carefully examined with results that are of considerable interest. It was found that in normal tadpoles the 4 protozoa studied were almost entirely limited to the rectum. One or 2 Opalinae were observed in the intestinal contents of 7 of the 25 control specimens; these were probably transients and not regular inhabitants of this part of the digestive tract. Hexamitus in considerable numbers was encountered in 1 tadpole and *Giardia agilis* occurred in many of the tadpoles. In the intestine of the thyroid-fed tadpoles, on

the other hand, there was an incidence of infection of 100 per cent. with Opalina, and an average of 1.94 per field; 100 per cent. of infection with Hexamitus, many being present in all but 3 specimens; and 25 per cent. of infection with Trichomonas, although very few in numbers. Examinations of various parts of the intestine revealed a rather constant number of the various species throughout. A comparison of the effects of thyroid feeding and of natural metamorphosis reveals the fact that in both cases the Opalinae are similarly distributed. These data indicate that changes in the intestine due to a thyroid diet rather than the direct action of the thyroid substance has caused an emigration of the

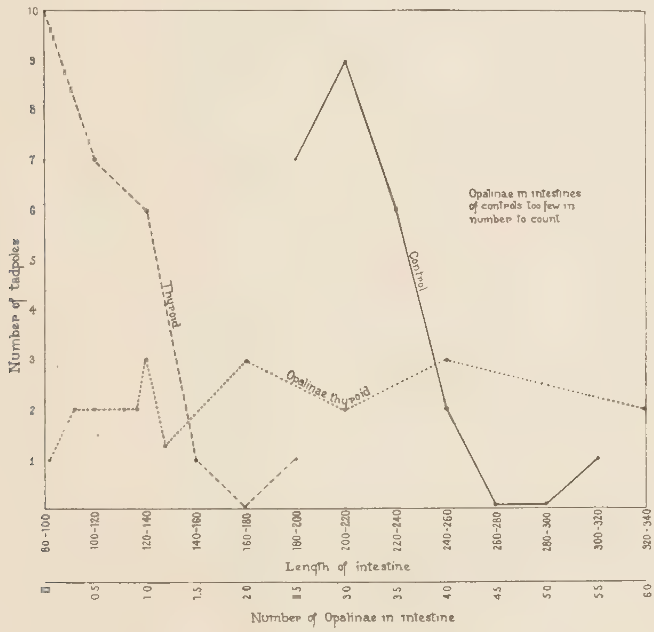

Fig. 2.—Curves showing the length of the rectum and comparative numbers of Opalinae present in 25 control and 25 thyroid-fed tadpoles of the green frog.

protozoa from the rectum and their distribution throughout the intestine, which is not their normal habitat.

5. *Experiments with an Animal Diet.*—Metcalf (1920, in litt.) has suggested that the disappearance of Opalinae in the green frog might take place "at the time of change from vegetable to animal diet." Tadpoles are essentially vegetable feeders but will take animal food when available. They will devour dead animals that they may find in their habitat and will feed on animal food entirely in the laboratory. It has been shown above that the Opalinae disappear from the tadpoles during metamorphosis but before transformation is completed. At this

time the diet appears to be the same as that of the younger tadpoles, the change to the purely animal diet not occurring until the young frog leaves the water. The infection thus disappears before the food becomes animal in nature. My experiments designed to test the effects of an animal diet have not been conclusive. Tadpoles of the green frog, when fed on dessicated glandular substances, have been shown above to lose their infections with Opalinae entirely or to be infected with lesser numbers. Beef meal was tried as a food in one experiment but most of the tadpoles died within 3 days. The 3 that survived were all infected with Opalinae, but the numbers of these were less than in control tadpoles. Twenty-five tadpoles of the green frog were fed on a diet consisting of the following ingredients: liver, 35 per cent.; beef, 27; dry ox blood, 18; lard, 20; and calcium carbonate, 1.5. A similar diet has been found by McCollum and Simmons to be favorable for the growth and reproduction of rats. These tadpoles, as shown in Table 3, did not exhibit any great decrease in the incidence of infection nor the numbers present of Trichomonas and Hexamitus. Unfortunately the controls were very lightly infected with Opalina and Nyctotherus and hence no conclusions could be reached regarding these forms.

A suggestion has been made to me by Metcalf (1920, in litt.) to account for the presence of Opalinae in the tadpoles and their absence from the adults of the green frog, namely, that "the tadpoles of *R. clamitans* may become infected with cysts of a species of Opalinid not found in the adult *R. clamitans* and that they thrive for a time and later in the life of the host disappear." This suggestion may be tested by experiment. Metcalf, indeed, "was able to infect several species of Anura with cysts of unaccustomed Opalinids and the Opalinids throve in the tadpoles of the unaccustomed hosts for a period of about 4 months." The tadpoles had to be killed at the end of this period. Since adults of the green frog do not usually harbor any species of Opalina, any species that occurs in the tadpole of this species of frog would be one "not found in the adult." The difference between the green frog and certain other species of frogs and toads is that no species of Opalinae have become regular inhabitants of the intestine of adults of the former, whereas, each of the latter is infected by one or more species, but refractory toward other species. The problem is the fundamental one of specificity in parasitism.

SUMMARY

1. The study of the incidence, distribution and numbers of the ciliate Opalina, in the intestine and rectum of the tadpoles of *Rana clamitans* indicates (a) that these tadpoles are very susceptible to infection with

Opalinae, the ciliates being numerous in specimens from 10 days old to the time of metamorphosis; (b) that during early stages of metamorphosis Opalinae are numerous in the rectum but not in the intestine; (c) that during intermediate stages part of the Opalinae migrate into the intestine, and (d) that in late stages all of the Opalinae disappear from the rectum and later from the intestine. Thus no young green frogs are infected with this protozoon and no species of Opalina seems to have succeeded in maintaining itself in the intestine or rectum of adult frogs of this species.

2. The ciliate, Nyctotherus, and flagellates, Trichomonas and Hexamitus, were found in all stages in the metamorphosis of green frog tadpoles and infection with these protozoa is probably continuous from tadpole to adult.

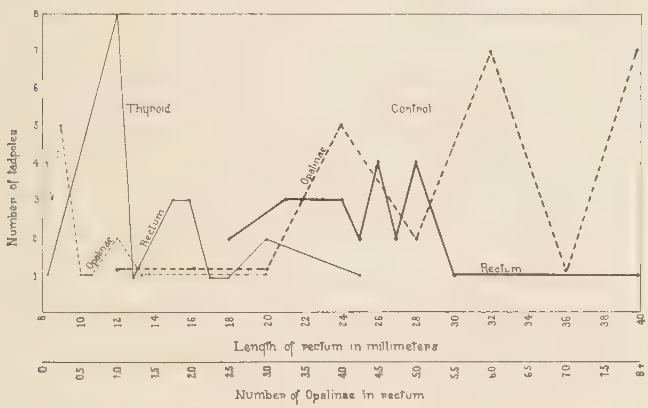

Fig. 3.—Curves showing the length of the intestine of 25 control and 25 thyroid-fed tadpoles of the green frog, and the number of Opalinae in the intestine of the latter.

3. Both adults and tadpoles of *Rana pipiens* and the toad are infected with Opalinae. This infection is continuous from tadpole to adult, and not an infection of the tadpole which disappears during metamorphosis and is followed by a reinfection of the adult.

4. Lack of food is disadvantageous to the intestinal protozoa of tadpoles of the green frog, leopard frog, and toad. Opalina was rarely present after a period of two weeks; Nyctotherus disappeared usually in about 1 week; and Hexamitus, although persisting in considerable numbers in toad tadpoles, decreased in both *Rana pipiens* and *R. clamitans.*

5. Preliminary experiments in 1921 on the effects of desiccated thyroid substance on the intestinal protozoa of green frog tadpoles

showed that considerable mortality occurs after one week. During this period external metamorphosis advances rapidly and the length of the intestine decreases about 50 per cent.

6. Further experiments in 1921 with desiccated thyroid, thymus, ovaries and orchic substance show that thyroid feeding results in the decrease both in incidence and numbers of Opalina, Nyctotherus, Trichomonas and Hexamitus. The 2 holozoic forms, Nyctotherus and Trichomonas, undergo a decrease in incidence but an increase in numbers when their tadpole hosts are fed on thymus, ovarian, or orchic substance. The 2 forms with osmotic nutrition, Opalina and Hexamitus, decreased in both incidence and numbers in tadpoles fed on these 3 substances.

7. Experiments in 1922 with thyroid, prostate, pituitary, suprarenal and orchic substances were only partially successful because of the low incidence of infection with Opalina and Nyctotherus. Trichomonas was adversely affected noticeably by diets of thyroid and prostate substance, both as regards incidence and numbers present and was reduced slightly in tadpoles fed on suprarenal substance. Very little difference was produced by diets containing pituitary and orchic substances. Hexamitus was influenced adversely very slightly by prostate and considerably by thyroid substance. An actual increase in numbers of Hexamitus was recorded in tadpoles fed on pituitary, suprarenal and orchic substances and an increase in incidence in tadpoles fed on pituitary and orchic substances. It is interesting to note that the 2 substances that are known to bring about marked modifications in the intestine, namely, thyroid and prostate, affect adversely the intestinal protozoa.

8. Further experiments in 1922 with thyroid substance furnished more extensive data. After thyroid feeding for 4 or 5 days Nyctotherus was almost eliminated from the rectum; Opalina decreased 16 per cent. in incidence and very markedly in numbers; Trichomonas decreased 80 per cent. in incidence and only a few were present; and Hexamitus decreased 8 per cent. in incidence and very greatly in numbers. The intestines of the control tadpoles were almost entirely free from protozoa, but in the thyroid-fed animals the intestine of every specimen was heavily infected with both Opalina and Hexamitus and 25 per cent. were infected with Trichomonas. Apparently the changes in the alimentary canal due to thyroid feeding brought about a change in distribution of these protozoa similar to that found in tadpoles undergoing metamorphosis. This adds weight to the hypothesis that it is not the direct effect of the food of the host but the changes in the digestive tract due to this food that influence the incidence and numbers of the protozoa present.

9. Tadpoles of the green frog were fed on an animal diet that has been proved to be satisfactory for the growth and reproduction of rats. No marked changes in the incidence and numbers of Trichomonas and Hexamitus were induced by this diet.

Literature Cited

Gudernatsch, J. F. 1912.—Fütterungsversuche an Amphibienlarven, Zentralbl. f. Physiol., 26 : 323-325.

1914.—Feeding Experiments with Tadpoles. Amer. Jour. Anat., 15 : 431-480.

Hegner, R. W. 1922.—The Effects of Prostate Substance on the Metamorphosis of the Intestine of Frog Tadpoles. Amer. Jour. Physiol., 61 : 298-299.

Kudo, R. 1922.—On the Protozoa Parasitic in Frogs. Trans. Amer. Micr. Soc., 41 : 59-76.

Shumway, W. 1917.—The Effects of Thyroid Diet upon Paramaecium. Jour. Exp. Zool., 22 : 529-563.

Swingle, W. W. 1918.—The acceleration of Metamorphosis in Frog Larvae by Thyroid Feeding, etc. Jour. Exp. Zool., 24 : 521-543.

PHASES IN THE PARASITISM OF THE UNIONIDAE

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There are few cases, probably, in which an animal parasite is itself an article of commerce. The large demand for mother of pearl in the industries has led to the artificial propagation of the fresh-water mussel, remarkable for its parasitic larval development. Although the parasitic stage is of comparatively short duration, it is an essential step in the life history. A thorough understanding of this as well as of other stages has been a prerequisite for successful propagation.†

The whole problem of mussel propagation has proved more complex than it was at first supposed to be. In early attempts (Lefevre and Curtis, 1912) at artificial infections of fish with the glochidia of mussels a susceptibility of certain groups was recognized, but the relation of one mussel as a specific parasite of a given fish or group of species was not recognized. The acquirement of this information made possible the propagation of more species of mussels, among them some of the most valuable commercial varieties. Another barrier to success was the subsequent post-parasitic stage in the mussel, called the juvenile, which on account of its minute size and vulnerability seldom survived. A method of bringing it through this critical period was required. Steps toward the attainment of the desired goal and a discussion of this phase of the life history have been made the special subject of other papers. In the progress of the investigations and the propagation of mussels opportunities for observation of the parasitic stages are continually presented which, while aiding the development of mussel culture, may likewise contribute features of interest to the parasitologist.‡

PREPARASITIC PERIOD: GLOCHIDIUM. MEANS OF INFECTION

The glochidium or parasitic larva developing from the egg within the modified gills or marsupium of the parent mussel is expelled, in the case of "summer breeders" (tachytictic forms), at least, soon after maturity. In the case of the winter breeders (bradytictic) the larvae after maturity (?) are retained for months over winter to be discharged

* Contribution from the U. S. Fisheries Biological Laboratory, Fairport, Iowa. Published with permission of the Commissioner of Fisheries.

† For full details regarding the progress of experiments in mussel culture reference may be made to recent summaries and bibliographies (Coker, Shira, Clark, and Howard, 1921; Howard, 1922).

‡ Acknowledgement is hereby made of the assistance of Dr. R. E. Coker and Mr. R. L. Barney who read the manuscript and offered valuable suggestions, and to Mr. J. B. Southall for assistance with the photographs.

in the spring or summer. The two-valved form of the glochidium is adapted for attachment to a host. Among glochidia two general types are recognized, the hooked and the hookless; for the most part the hooked glochidia are larger and adapted for attachment to the exterior of fishes, as to fins, soft scaleless parts or even to the epidermis covering the scales, whereas the hookless type are smaller and become attached more readily to the minute lamellae or to the filaments of the host's gills.

The glochidia after extrusion from the parent mussel have been observed falling to the bottom in still water. In flowing water they may be carried in suspension for long distances, as shown by their occurrence in plankton catches (Kofoid, 1903, 1908). This transference seems to be due to their small size perhaps more than to specific gravity; as regards American species, so far as known, it is not due to any power of self-propulsion, notwithstanding early accounts frequently seen repeated in text books, of their locomotion through flopping of the valves after the manner of the scallop (Pecten); however, Japanese species are said to possess this power (Arey, 1921: 470, footnote). The mere presence in the water of the hookless type of glochidia, especially the smaller species, subjects fish to the chance of infection; this results from their ready transference by currents and coming in contact with the gills of fishes through the latter's respiratory movements.

The high percentage of infections and the heavy individual infections of some species indicate a more or less definite ecological relation between host and parasite (Coker, Shira, Clark and Howard, 1921). This relationship is supposed to exist between the river herring, *Pomolobus chrysochloris* Raf. and the niggerhead mussel, *Quadrula ebenus* Lea. Both are found in swift water habitats which would favor the carriage in suspension of the small glochidium of *Q. ebenus*. Other illustrations are the channel catfish, *Ictalurus punctatus* Raf., and the wartyback mussel, *Quadrula pustuolsa* Lea; the small mouthed black bass, *Micropterus dolomieu* Lacépède (not the sole host), and the mucket, *Lampsilis ligamentina* Lamarck. During the spawning * season (May) of the latter mussel, seine catches of the bass in Fox River have revealed a 90 per cent. infection. No doubt the spawning habits of the bass making their nests in the swift stony shallows brings them into closer association. The hooked glochidia seem less dependent upon the buoying action of currents. Their attachment in infections is usually upon fins, no doubt the result of near approach of the fish to the bottom where through the agitation produced by the fins they may be carried upward into contact with the host.

The presence of the "larval thread" (Lefevre and Curtis, 1912: 151) in hooked glochidia is thought by some to aid infection. The very

* Spawning in this case signifying extrusion of the glochidia.

large hookless glochidia of *Quadrula heros* Say have been observed in nature more commonly in external infections upon the fins than upon the gills. This may be correlated with the presence of a thread gland in this species, also (Howard, 1914) with their large size. The hooked glochidia of *Alasmidonta marginata* Say may be an exception to the rule of external infection, having been found in many cases upon the gills, but upon more substantial parts as the gill rakers and rachises of the filaments rather than the lamellae. One aid to infection is doubtless the natural association of fish and mussels on mussel beds, localities favorable to both (Howard, 1914: 39; Coker, Shira, Clark and Howard, 1921). In the case of mussel-eating fish, and especially the fresh water drum, *Aplodinotus grunniens* Raf., the glochidia from the parent mussels consumed may infect the predator. In fact, the drum is found commonly to be heavily infected by the glochidia of the thin shelled and easily crushed species *Lampsilis gracilis* Barnes, and *L. laevissimus* Lea, inhabitants of quieter water which because of absence of current must be less effective in distribution of glochidia. A close habitat association is seen between the Salamander mussel *Hemilastena ambigua* Say and *Necturus maculosus* Raf., the mussel in spawning season, at least, living on the stream bed beneath flat rocks, a place frequented also by the salamander (Howard, 1915).

Evidence of special means of attraction between parasitic species and host has been secured (Coker, Shira, Clark and Howard, 1921: 85, 120). This consists in *Lampsilis ventricosa* Barnes in elaborately developed and brightly colored mantle lobes in the gravid female. "These lobes in their form and coloration including an eyespot, resemble small fish, and the motion of these in the current still further enhances the resemblance. The enlarged marsupia distended with glochidia lie close to these flaps, one on each side. It has been suggested that a fish darting at this tempting bait may cause the extrusion of the glochidia and then become infected."

Later observations reveal a similar development in the closely related *Lampsilis luteola* Lam. which here accompanies the highly specialized condition of the marsupium presented throughout the Lampsilinae. In mussels brought from Lake Pepin during May (1921) and placed in aquaria this phenomenon was especially well displayed and to a greatly varying degree. Its high development in some individuals seemed to warrant special attention. The mussels when observed were in a cement aquarium in running water at a temperature of 73 F. Although not imbedded in the mud as they would normally appear in nature, their position was considered especially favorable for showing photographically the detail of the structures (Fig. 1). The enlarged portion or mantle lobe is ventral to the incurrent siphonal opening. It consists of a narrow dorsal (with respect to the mussel) portion with tenninal

eye-spot and broad ventral part with elaborately fimbriated edge, forming the tail of the simulated minnow (?). The coloration is striking. On a general ground color of reddish, are dark bluish or purplish bands with pencilings of black. The eye-spot is black with a light border. Extending out between the right and left mantle lobes in the region of the incurrent siphonal opening the light colored marsupia heavily distended with glochidia become visible. If undisturbed they are extended a full quarter of an inch beyond the mantle and a half inch beyond the shell.

Certain movements and reactions were noted. Regular undulations of two rapidly succeeding waves occurred lasting two seconds and each taking approximately a second to pass from the outer ventral lobe to the eye-spot. The interval between the undulations was timed and found to be somewhat uniform, as indicated by the following readings in seconds: 5, 5, 4, 5, 3, 5, 4, 4, 5, 4, 5, 5, 5, 4, 4, 4, 7, 4, 5, 4; average = 4.5 seconds. Withdrawal of the marsupium always followed the slightest disturbance of the water and invariably before the mantle lobes made any response. Motion of a small object like a pencil within the water at a distance of two inches caused retraction of the marsupium without visibly affecting the mantle lobes. No withdrawal followed light touching of the marsupium itself or the outer mantle lobe. Touching of the tentacles in the vicinity of the incurrent siphon promptly brought about the reaction. Considerable jar, as from a blow to the concrete tank, was not effective. Evidently the stimulus causing the reaction (withdrawal) was received through the tentacles. The question as to the function of these movements and reactions arises. The regular undulations suggest aid to respiration (for the enclosed young) but might also serve to attract a predatory fish as a possible host, suggested above.

The withdrawal of the marsupium and the closing of the valves, besides protecting the mussel, would cause extrusion of some glochidia with obvious advantage if in the presence of fish. It is significant perhaps that these two species (*Lampsilis ventricosa* and *L. luteola*) are predominately inhabitants of lakes or lacustrine portions of streams where dissemination by current action would be slight, and that the hosts of these mussels are predacious fish (chiefly members of the Centrarchidae and Percidae).

VICISSITUDES AND ENEMIES

With no known means of propulsion, dependent chiefly upon currents for transfer, the chances of the larva's finding the proper host must be exceedingly small (Lefevre and Curtis, 1912). Their tremendously prolific production contrasted with the relatively small

number found as parasites bears witness to the great mortality occuring in nature at this stage. Falling to the bottom they become the prey of such observed enemies as the widely distributed *Chaetogaster diaphanus* (Fig. 2) and the Rhabdocoels (Fig. 4). Of the latter *Stenostoma giganteum* Higley has been identified, and there are no doubt other predatory forms. Unequipped, so far as known, for independent existence the duration of their effectiveness must be brief. The longest observed survival, in the authors' experience, was one week.

It is a common observation in experiments of the authors that the glochidia meet with resistence to attachment on the gills in the case of the gars (Lepisosteus Lacépède), through the copious production by the fish of a protective mucus. Similar means of resistance may be more common than is at present known. In practical propagation this can be obviated by changing the water which holds the fish.

According to Arey (1921), attachment is controlled by contact stimulus solely. This being the case, it is mere chance whether right or wrong host is secured; if the wrong host, destruction by desquamation, cytolysis or phagocytosis or other immunizing agent ensues (Howard, 1914; Reuling, 1919). Destruction also follows attachment to a natural host which has acquired immunity (Reuling, 1919) by having had successive previous infections. The precariousness of this period for the potential parasite is realized from a contemplation of the pitfalls enumerated. Artificial infection successfully bridges over this critical period. A measure of effectiveness of artificial infection is the high utilization of the glochidia from a given mussel. In artificial infection 100 per cent. of the fish are infected, utilizing as high as 50 per cent. of the glochidia supplied; in nature the records give as small as 3.5 per cent. of 674 fish examined (Surber, 1913), and these but lightly infected. It is common in propagation operations to put 2,500 glochidia on a medium sized fish which in nature would commonly have at the most, perhaps, 250.

PARASITIC PERIOD

The parasitic period begins with the implantation of the glochidium. Contact of the host's tissues with the sensory hairs inside the widely gaping valves of the glochidium (Arey, 1921) causes the valves to close vice-like upon the gill or epidermis of the fins or body surface. These tissues react by enclosing the glochidium in a cyst, the complete implantation usually occupying only a few hours. The demonstration by Arey of the effectiveness of a mechanical factor by employing a minute aluminum clamp in this process seems to disprove the previously accepted belief in a chemical agent based chiefly on the response of glochidia to certain ions.

The encystment varies with the species, the encapsulating tissue being more extensive in some than in others. In the case of fishes having gills with thin lamellae the cyst formation may involve several of these. Following encystment the parasite undergoes changes in the nature of a metamorphosis. This consists in a transformation from a comparatively simple glochidial larva to the more complex juvenile with most of the organs of the adult.* Having completed its metamorphosis the young mussel makes its escape, extrication apparently being initiated by its own activity with possible reaction by the host.

DEGREE OF PARASITISM

Three distinct types of parasitism have been observed in freshwater mussels.

First, limited parasitism without growth.

Second, extended parasitism, or long continued demand upon the host, with more or less extended growth.

Third, abandoned parasitism, more or less complete loss of the parasitic habit.

The common relation between the parasitic mussel and its fish host is that of a limited parasitism (Fig. 5). The glochidium passes through a metamorphosis, making no external growth, and therefore probably receiving little sustenance from its host. The fresh-water mussels of commerce belong to this class.

Among some of the thinner shelled species of mussels the second type of parasitism prevails (Figs. 6 and 7). The parasite instead of leaving the host with the minute glochidial shell only, while still upon the fish, begins a growth which in some cases brings it to a size five times greater in linear dimensions than the glochidium. This is well illustrated by *Lampsilis laevissimus* Lea, *L. gracilis* Barnes, *Plagiola donaciformis* Lea, and *P. elegans* Lea (?) which all have small glochidia and have for a host the fresh-water drum, *Aplodinotus grunniens* Rafinesque. It has been suggested that this extra-glochidial growth is correlated with their small size, bringing them by compensatory growth to the size of other mussels when beginning the juvenile period. In the case of the large celt-shaped glochidia of *Lampsilis alata* Say and of *Lampsilis purpurata* Lamarck the growth on the host is limited to an extension sufficient only to give the form of the definitive shell (Howard, 1914). Evidence from the juvenile shell of *Lampsilis capax* Greene shows that it also belongs to this group, but its host (probably *A. grunniens*) has as yet not been reported. A single instance of such growth other than on the drum has been observed upon the mud cat,

* Among recent contributions on this phase perhaps the most complete is that of Herbers (1913), which gives a complete review of the literature.

Leptops olivaris Raf.; the mussel has been provisionally identified as *Quadrula lachrymosa* Lea, which also possesses a small glochidium.

The third type is represented by *Anodonta imbecillis* Say, with possibly closely allied species and by members of the genus Strophitus (Simpson). In *Anodonta imbecillis* (Fig. 3) the glochidia at maturity possessing the hooks characteristic of their parasitic relatives, the other Anodontas, instead of attaching to a fish continue their development within the marsupium of the parent. On completing metamorphosis they leave the parent mussel without extra growth and take up an independent existence as rapidly growing juveniles.

A similar modification of normal parasitism has been reported for *Strophitus edentulus* Say from a single instance noted (Lefevre and Curtis, 1912). Howard (1914) followed through normal parasitic development of this species on different fish hosts. If both types of parasitic behavior exist in this species, as reported, it presents an instance of facultative parasitism, or a condition intermediate between the first and third types.

DURATION OF PARASITISM

The period of parasitism extends from the time of implantation of the glochidium to the time of escape of the young mussel from its host. The length of this period shows considerable variation and, since it is a matter of some importance in mussel culture, has received considerable attention. Investigations have ascribed the variations to the operation of various factors which may be enumerated as follows: (1) temperature, (2) species of mussel, (3) species of host, (4) condidition of host, (5) age of glochidia, (6) nutrition with reference to location; these will be considered seriatum. References to earlier literature may be found in Lefevre and Curtis (1912).

Temperature was early recognized as a factor in determining the length of the parasitic period. Schierholz and Harms maintained that the duration of this period varied inversely with the temperature of the water. Lefevre and Curtis (1912: 167) recognized the modifying influences of obscure factors. A later publication (Coker, Shira, Clark, and Howard, 1921: 150) stresses the possibility of the operation of factors other than temperature. In recent experiments by the authors, a method was employed which, as far as known, eliminated other factors. Gars (Lepisosteus Lacepede) infected with the glochidia of *L. anodontoides* Lea on June 22, 1921, were divided into two lots and kept in separate troughs at temperatures that differed by approximately 7.3 degrees, averaging 81.3 F. and 74 F., respectively (average of three daily temperatures for 18 and for 22 days, respectively). The lower temperature was obtained by conducting the water through an underground cistern. Those held at higher temperature completed the period

of parasitism in less than 18 days, those at lower temperature in more than 22 days, a difference of 4 days, or 22 per cent. Such are the reactions under conditions of summer temperature.

On falling temperatures, as at the approach of winter, the encysted mussels remain upon the host until the following spring, when, with increase of temperature, they make their escape. Many observations on shedding have called attention to the possibility that retaining and shedding of glochidia may both be governed by more or less fixed points on the temperature scale. Data have been obtained which give some

TABLE 1.—WATER TEMPERATURES ACCOMPANYING THE SHEDDING AND THE RETAINMENT OF JUVENILES OF *LAMPSILIS LUTEOLA* LAM. BY THE LARGE-MOUTHED BLACK BASS, *MICROPTERUS SALMOIDES* LACÉPÈDE

Date	Temp. Water, ° F.	Date	Temp. Water, ° F.
August 31, 1921*a*	77	November 28	39*d*
September 1	79	December 1	40
September 2	78	December 7	39
September 3	79	December 14	38
September 4	79	December 30	33
September 5	79	January 1, 1922	33
September 6	76	January 7	33
September 7	76	January 14	33
September 8	73	January 21	33
September 9	73	January 30	33
September 10	72	February 1	33
September 11	76	February 7	33
September 12	72	February 14	33
September 14	73	February 21	33
September 15*b*	75	February 28	33
September 29*c*	71	March 1	33
September 30	65	March 7	33
October 1	64	March 14	35
October 3	62	March 21	35
October 4	62	March 28	42
October 5	58	April 1	44
October 7	56	April 7	57
October 11	54	April 14	52
October 12	54	April 19	52
October 17	61	April 28	46
October 21	56	May 1	61
October 25	56	May 7	63
November 2	60	May 10	64*e*
November 7	50	May 12	67*f*

a Date of fourth infection; all during same season, 1921.
b Date on which fourth infection was completely shed; note temperature.
c Fish infected for fifth time.
d Lowering temperatures.
e Apparently shedding begun.
f Shedding very evident.

support to this view, and help to determine these temperatures. Further evidence is necessary to substantiate these findings.

The data given in the following tables (1 and 2) were taken from experiments not otherwise falling within the province of this paper. The fish referred to in Table 1 had received successive infections; that of Table 2 received a single infection October 27, carrying the glochidia through the winter and yielding live juveniles in the spring. Table 1 shows dates and temperatures at periods of infection and shedding during September. A subsequent infection of the same fish shows

retainment upon falling temperature. It will be noted that following September 29, the date of the second infection, the temperatures fell below 65 F.; shedding occurred the following spring upon the attainment of approximately this temperature.

Table 2, represents a single infection and shedding. During the period of retainment, at no time did the temperature go above 65 F. At this temperature in the spring shedding began. These observations seem to fix the "critical" temperature as near 65 F. Whether this temperature is "critical" for the metabolism of the host, or of the mussel, or of both, has not been determined.

In nature it is not uncommon for the mussel parasites to be carried over winter, and fall infections seem to be normal for certain species.

TABLE 2.—WATER TEMPERATURES ACCOMPANYING THE RETAINMENT AND THE SHEDDING OF JUVENILES OF *LAMPSILIS LIGAMENTINA* LAM. BY THE LARGE-MOUTHED BLACK BASS, *MICROPTERUS SALMOIDES* LACÉPÈDE

Date	Temp. Water, ° F.	Date	Temp. Water, ° F.
October 27, 1920	48	November 17	45
October 28	48	December 20	39
October 29	47	December 22	38
October 30	50	January 3, 1921	39
October 31	51	January 21	38
November 1	42	February 13	38
November 2	42	April 15	55
November 3	43	April 18	49
November 4	46	April 20	51
November 5	48	April 21	55
November 6	46	April 26	60
November 7	45	April 28	60
November 8	46	May 8	60
November 9	46	May 13	62
November 10	41	May 16	61
November 11	35	May 17	61
November 12	32	May 18	65*b*
November 13	32	May 20	72*c*
November 14	33	May 21	73*d*
November 15	34	May 23	76*e*
November 16	43.3, 40*a*		

a Temperatures indoor trough and outdoor pond, respectively, for comparison; temperatures previous to this date from pond, after same from indoor trough in which fish host was held.
b, c Juveniles being shed.
d Found juveniles in trough.
e Juveniles mostly gone from fish.

This is true of *Obovaria ellipsis* Say on the sturgeons, Scaphirhynchus Heckel, of *Quadrula heros* Say, on various species of fish, and of *Hemilastena ambigua* Say, on the salamander, *Necturus maculosa* Raf. This wintering on the host doubtless has distinct advantages to the mussel, chief among which is the earlier start obtained and consequent escape from the ravages of enemies during the most critical period in the life history.

Wintering over is apparently the normal condition in the extraglochidial parasitism of the mussels (list, p. 73) which parasitize the drum, *A. grunniens* Raf. If this is the case, glochidia are retained, in spite of summer temperatures, until spring of the following year.

A peculiar case of retention over winter and into summer (July 3) was observed. Gar fish were infected late in the fall with the yellow sand shell and their persistence after the "critical" temperature attracted attention; examination revealed the fact that all the mussels were dead. Two experiments of this sort are thought to indicate that the yellow sand shell is not normally carried over winter. The persistence of the dead parasites is added evidence that normal elimination is initiated by the parasite.

Harms noted a difference in length of parasitic period between Unio and Anodonta which he ascribed to the less advanced stage of development of the glochidium in Unio when leaving the parent mussel. Lillie (1895) working upon American species from the standpoint of embryology substantiated the above, finding greater development in Anodonta sp. than in Unio sp. (*complanata?*). He also seems to imply (p. 65) that development of the glochidium of Anodonta progresses during the winter while still in the brood pouch of the parent (see age of glochidium p. 78). These facts have a practical application in mussel propagation in that they point to the necessity of determining whether a glochidium is normally released in the fall or in the spring.*

The present authors have no specific data with reference to the European forms nor to the differences between hooked and hookless glochidia mentioned by Lefevre and Curtis. It is believed, however, that there is considerable variation in habit, at least, among both these large groups as suggested in the following examples.

The hooked glochidium of *Hemilastena ambigua* Say is normally a fall infecting species, having been observed both in artificial and natural infection to carry through, while *Anodonta imbecillis* has lost its parasitism. These instances indicate that all hooked glochidia are not like the Anodonta reported by Lillie. As between summer and winter breeders (both within the hookless group) it has been noted, as far as they have been compared, that the summer breeders have the shorter parasitic period. No extensive investigation of the groups, as a whole, has been made, but the difference was noted incidentally on several occasions when infections with the two groups of mussels corresponded closely in time.†

In later experiments when opportunity presented, this phenomenon was given closer attention. The following experiments demonstrate the difference quite clearly. Gars (*Lepisosteus platostomus* Rafinesque) were infected on July 12 (1918) with *Quadrula plicata* Say (a "summer breeder"). The same species of fish on July 13 were infected with

* *Lampsilis anodontoides* is a doubtful case. Although infection upon a fish can be secured in the fall none thus far tried have survived the winter upon the host (see duration of parasitism p. 74).

† Some of the records pertaining to such observations were lost with the burning of the Fairport Laboratory in December, 1917.

Lampsilis fallaciosa Smith (a "winter breeder") and kept in the same pond. On July 23 the *Q. plicata* had all been shed, while the *L. fallaciosa* were still present in the full number, complete elimination not being observed until July 31. In the first case a period of parasitism of 11 days or less, in the second one of 4 to 7 days longer was indicated. In another experiment the period of parasitism for *L. anodontoides* on the gar was 21 days (July 7 to July 28, 1919); catfish infected with *Q. pustulosa* on July 18, 11 days later, under otherwise similar conditions, were also free of the parasites on July 28, or after a period of 10 days, less than one-half that required by *L. anodontoides.* A better test of this phenomenon would be a simultaneous infection of the same individual host by members of the two groups, for example, *Q. plicata* and *L. anodontoides* on the gar. Another example is the extended parasitism of all known species but one infecting the drum, *A. grunniens,* in which the greater development demands more time, namely, nine months or more as compared with two or three weeks for the one exception, *Plagiola securis* Lea.

Corwin (1920) finds a difference in the length of parasitism of the same mussel on different hosts. Mussels were shed 5 to 10 days earlier by the small-mouthed black bass, *Micropterus dolomieu* Lacépède, than by four other species infected at the same time. Our own observations give some indication of a shorter period for *Lepomis pallidus* Mitchill than for *Micropterus salmoides* Lacépède, when infected with *Lampsilis luteola* Lamarck.

Coker (1921: 150) has suggested that the vitality of the host may be a factor in affecting the length of the parasitic period. The only data bearing on this topic possessed by the present writers is the observation of mussels escaping from the gills of dead and dying hosts. These observations indicate that a moribund condition in the host hastens shedding when metamorphosis is well advanced.

Lampsilis luteola Lamarck is known to spawn out early in the summer (about July) in Lake Pepin; it is difficult to find gravid females at this time. Later in the summer a new brood of glochidia is produced. Corwin (1920) finds a difference in the results obtained from infections made with these two types of glochidia, which he calls "old" (carried by the parent over the winter and spring) and "new" (mature [?] ones of the current season). Since the two types may, during a short period, be obtained together, Corwin, carrying them in parallel experiments, found that the "old" glochidia completed the parasitic period in a shorter time than the "new." A difference of development in the "old" and "new" glochidia of one species is here suggested comparable to that seen in different species.

Considerable variation has been reported by a number of workers in the lengths of the parasitic period of "glochidia of the same species

when infected upon the same fish" (Coker, Shira, Clark, and Howard, 1921). This has been explained as being due to differences in nutrition as governed by position on the body of the host. Glochidia on the sides of the gill filaments are thought to make their escape before those on the tips, the cause supposedly being the better vascular supply in the position of the former. This condition is commonly noted in infections. In some cases the differences may be striking; Lefevre and Curtis have observed variations of from 13 to 24 days, or almost 100 per cent. Since variation may be due to the death of the glochidia, a condition frequently seen in normal infections, careful discrimination is required in coming to conclusions as to the causes of such variation in time.

RACIAL AND INDIVIDUAL IMMUNITY

Certain species of mussels in their parasitism are restricted to a single species or genus of hosts. In others is found the opposite extreme in which almost any species of fish seems to meet the requirements. When a glochidium becomes attached to a species to which it is not adapted (non-host) it becomes encysted apparently in the normal manner, but is sloughed off within two or three days. Well known examples of restricted parasitism are that of the wartyback mussel, *Quadrula pustulosa* Lea, on the catfishes, Siluridae, the niggerhead, *Quadrula ebenus* Lea, on the river herring, *Pomolobus chrysochloris* Raf., and the yellow sandshell, *Lampsilis anodontoides* Lea, on the garpikes, Lepisosteus Lacépède. A striking example of adaptation is that of *Aplodinotus grunniens* Raf. which is the sole known carrier of eight or more species of mussels. The known species are as follows:

Lampsilis (*Proptera* Ort.) *alata* Say.
Lampsilis (*Proptera* Ort.) *purpurata* Lam.
Lampsilis (*Proptera* Ort.) *laevissima* Lea.
Lampsilis (*Proptera* Ort.) *capax* Green (probable).
Lampsilis (*Paraptera* Ort.) *gracilis* Barnes.
Plagiola donaciformis Lea.
Plagiola elegans Lea.
Plagiola securis Lea.

As many as three or four different species have been observed at one time upon a single individual (Figs. 6 and 7). Racial immunity is best illustrated and readily recognized in such cases of restricted parasitism. In such instances any species that will not carry a given species of mussel may be designated as racially immune. The mechanism of racial immunity is probably similar to that of individual immunity (Reuling, 1919). A previously unreported case of racial immunity is that of the orange spotted sunfish, *Lepomis humilis* Girard, toward the mussel *Lampsilis luteola* Lam. This was unexpected in view of the fact that all of the five species of the Centrarchidae previously

tested had proved to be carriers of this mussel. An example of unrestricted parasitism is that of the hooked glochidium *Alasmidonta marginata* Say which was found in natural infections upon the following species of fish: *Moxostoma aureolum* Le Sueur, *Catostomus commersonii* Lacépède, *Catostomus nigricans* Le Sueur, *Ambloplites rupestus* Raf., *Chaenobryttus gulosus* Cuvier and Valenciennes. Another example is the large hookless glochidium of *Quadrula heros* Say reported as normally parasitizing as many as thirteen different species of fish (Coker, Shira, Clark, and Howard, 1921, Table 19).

When an individual fish has carried three or more broods of glochidia through the parasitic period it may not be further susceptible to infections from any species of mussel so far as known which means that attaching glochidia will be eliminated in a few days without metamorphosis. Such a condition in a fish may be termed individual immunity. The action has been described by Reuling (1919) as cytolytic, the parasite being first killed by a hemolysin and then eliminated by desquamation. The gross features of the process are similar to those of racial immunity described above.

SOURCES OF LOSS

In nature once the glochidium gains attachment to a suitable host the loss in numbers becomes, doubtless, relatively low. In the progress of experimental propagation of mussels, however, with the confinement of fish in small enclosures or in troughs losses are often very high, from diseases produced by Saprolegnia, *Bacillus columnaris*, Ichthyopthirius, and similar organisms. Methods for the treatment of these organisms have been devised. A certain amount of handling of the fish is necessary, during which abrasions to the skin become points of entrance for microorganisms of which *B. columnaris* is most virulent at the season when the greater number of experiments are conducted. Excessively heavy infections are often the source of loss.

Recent experiments conducted by the authors gave opportunity to test the effectiveness in artificial infection experiments of a mode of treatment for *Bacillus columnaris* devised by H. S. Davis (unpublished report) for general treatment of fish affected by this organism. The experiments mentioned involved the use particularly of the species *Micropterus salmoides* Lacépède and *Lepomis pallidus* Mitchill. The fish were introduced into experimental troughs on June 21 (1921). By the end of the fourth day 18 out of 95 fish had succumbed from the disease. Treatment with copper sulphate after the manner recommended by Davis * was begun on June 25 and repeated on June 30,

* The fish were subjected for two to three minutes to a solution of copper sulphate, 1 : 1,000 dilution.

July 2, 6 and 9. Immediately following the first treatment marked improvement was noted; during the period between the first and second treatment only 4 out of 74 fish died; between the second and third, 4 out of 69. The results are striking in view of the fact that the disease had gained a foothold so that the treatment was therapeutic. It is a point of interest that the copper sulphate treatment had no apparent effect on the encysted glochidia, since the fish from which the highest production in juvenile mussels was obtained had been treated the full number of times. As a further means of ensuring sanitary conditions for fish retained as hosts in small enclosures care must be taken to remove all uneaten organic material, and to guard against the introduction of infective forms carried by the small fish supplied as food. In this connection it is worthy of note that feeding of fish was successfully dispensed with in the experiments referred to above.

References Cited

Arey, Leslie B. 1921.—An experimental study on glochidia and the factors underlying encystment. Jour. Exp. Zool., 33: 463-499; 3 pl.

Coker, R. E.; Shira, A. F.; Clark, H. W., and Howard, A. D. 1921.—Natural history and propagation of fresh-water mussels. Bull. U. S. Bur. Fish., 37: 77-181; 17 pls.

Corwin, R. S. 1920.—Raising fresh-water mussels in enclosures. Trans. Amer. Fish. Soc., 49: 81-84.

Herbers, Karl. 1913.—Entwicklungsgeschichte von *Anondonta cellensis* Schröt. Zeitschr. wiss. Zool., 108: 1-174; 104 fig.

Howard, A. D. 1914.—Experiments in propagation of fresh-water mussels of the Quadrula group. Rept. U. S. Com. Fish., App. IV, 52 pp., 6 pls.

1915.—Some exceptional cases of breeding among the Unionidae. Nautilus, 29: 4-11.

1922.—Experiments in the culture of fresh-water mussels. (In press.)

Kofoid, C. A. 1903.—The plankton of the Illinois River. Part I. Bull. Ill. State Lab. Nat. Hist., 6: 95-629.

1908.—Part II. 8: 1-361.

Lefevre, George, and Curtis, W. C. 1912.—Studies on the reproduction and artificial propagation of fresh-water mussels. Bull. U. S. Bur. Fish., 30: 105-201; 12 pl.

Lillie, F. R. 1895.—The embryology of the Unionidae. Jour. Morph., 10: 1-100; 6 pl.

Reuling, F. H. 1919.—Acquired immunity to an animal parasite. Jour. Inf. Dis. 24: 337-346.

Surber, T. 1913.—Notes on the natural hosts of fresh-water mussels. Bull. U. S. Bur. Fish., 32: 101-116; 3 pl.

Explanation of Plate VII

Fig. 1.—A gravid female of *Lampsilis luteola* Lamarck, showing mantle lobe and extruded marsupium (light, regularly striated structure).

Fig. 2.—*Chaetogaster diaphanus* Gruithuisen, an observed predator upon mussel glochidia.

Fig. 3.—Juvenile of *Anodonta imbecillus* Say. Removed from the marsupium of the parent July 15. An illustration of abandoned parasitism. The foot and the gill papillae are visible, thus distinguishing it from a glochidium.

Fig. 4.—*Stenostoma giganteum* Higley. The specimen photographed has ingested 8 glochidia which are visible as lighter rounded areas.

Fig. 5.—*Lampsilis fallaciosa* Simpson on *Lepisosteus platostomus* Rafinesque (a natural infection). Illustrating limited parasitism, in which the parasite makes no external growth, with consequent slight demand upon the host.

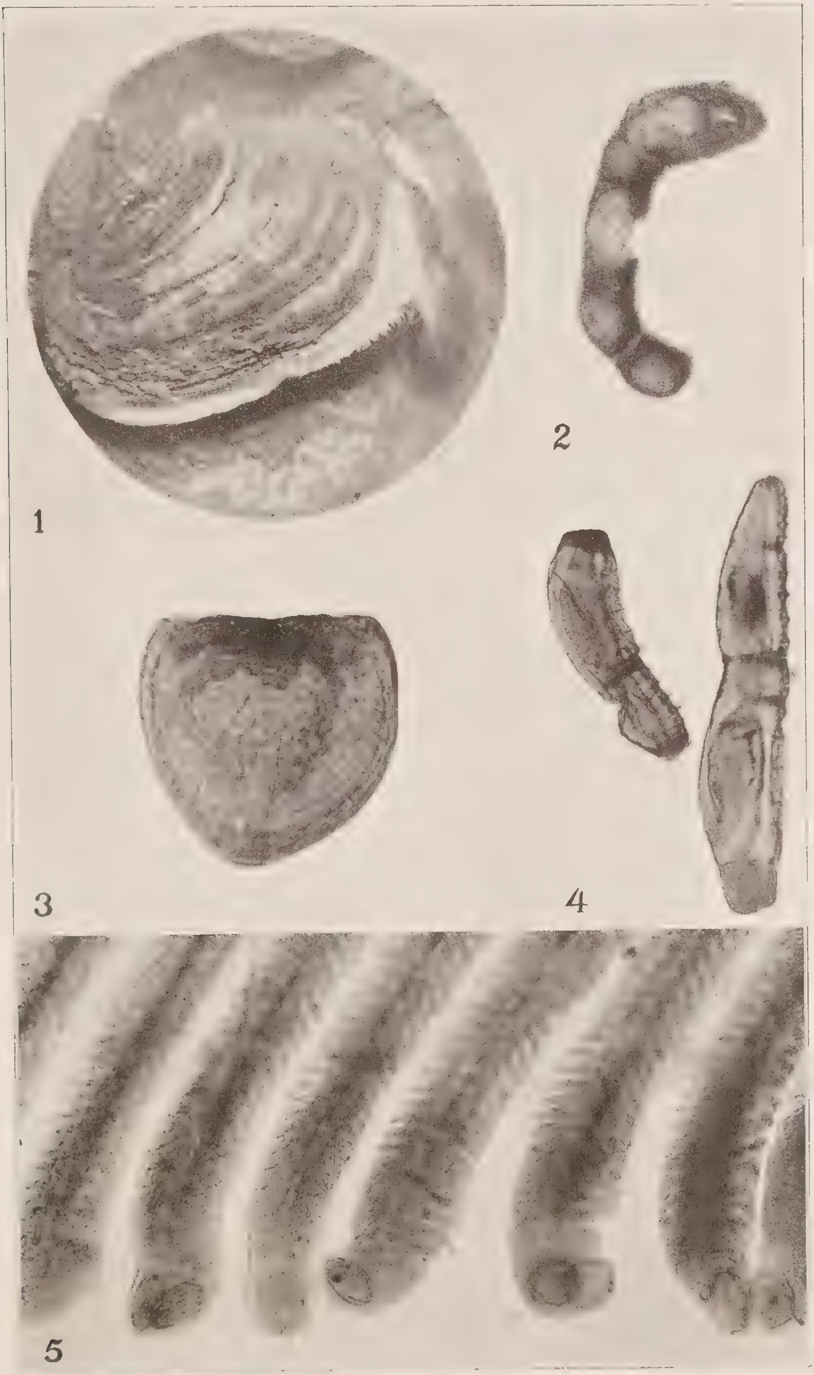

PLATE VII

EXPLANATION OF PLATE VIII

Fig. 6.—*Lampsilis alata* Say, *L. laevissimus* Lea, and *L. gracilis* Barnes on *Aplodinotus grunniens* Rafinesque. Illustrating extended parasitism, in which there is growth and increased demand upon the host.

Fig. 7.—*Lampsilis laevissimus* Lea and *L. gracilis* Barnes on *Aplodinotus grunniens* Rafinesque. Illustrating extended parasitism. The lower encysted mussel is *L. laevissimus;* the celt-shaped glochidial shell is visible in dorsal view as a saddle upon the definitive shell.

PLATE VIII

THE PREVALENCE OF HOOKWORM AND OTHER INTESTINAL NEMATODES IN ADULT FILIPINOS

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INTRODUCTION

During the collegiate year 1921-1922 students of the University of the Philippines at Los Baños were examined for intestinal parasites in connection with medical examination that is required of all matriculants at the university. The object of the examinations for parasites was to determine primarily the incidence and degree of infestation of normal adult Filipinos with hookworms and, incidentally, to obtain information on the degree and extent of infestation of these individuals with other intestinal parasites.

Studies on intestinal parasitism in the native population of the Philippine Islands are not novel undertakings. Since the American occupation of the Philippine archipelago a number of surveys have been made with a view to determining the incidence of protozoon and helminthic parasites in Filipinos and the bearing of such parasitism on public health. These surveys have been based, for the most part, on studies of inmates of Bilibid Prison, of patients in the Philippine General Hospital, on records of the medical departments of the army and navy and on records of autopsies performed in the College of Medicine and Surgery of the University of the Philippines. Several investigations on the prevalence of intestinal parasites in certain localities have also been published, but despite the fact that a considerable mass of data have been accumulated, no parasitological study based on the normal heterogenous adult population of the Philippines and carried out by modern methods has been published prior to this investigation.

The fact that the student population of Los Baños is typical of the population of the Philippine Islands in general was the chief reason why this study was undertaken. Forty-six provinces and subprovinces including the city of Manila are represented in this survey. The character of the student population at Los Baños is quite varied, embracing various economic classes in proportions approximating the relative proportions of these classes in the the general insular population. The data obtained in the course of this investigation may be considered,

therefore, a fair index to intestinal parasitism in adult Filipinos, despite the fact that the number of cases studied has not been very great.

The results of our study relating to infestation with cestodes and trematodes have already been published (Schwartz and Tubangui, 1922). The object of this paper is to present the results of our survey as regards the prevalence of intestinal roundworms.

METHODS

Samples of feces were submitted by students [1] who were furnished bottles containing about 50 c.c. of 10 per cent. formalin and were given directions to avoid contaminating the feces with soil and other objects. Each student was instructed to deposit his feces on clean paper and to place it, with the aid of a twig or clean stick, in the bottle containing formalin. This procedure proved altogether satisfactory because the preserving fluid eliminated possible dangers of infection as a result of

TABLE 1.—Results of Examinations for Intestinal Roundworms

		Percentage
Number Examined	649	.....
Number infected	550	84.76
Number infected with hookworm	316	48.69
Number infected with Ascaris	229	35.28
Number infected with Trichuris	357	55.00
Single infections	267	41.14
Hookworm	99	15.25
Ascaris	45	6.93
Trichuris	123	18.95
Double infections	214	32.97
Hookworm and Trichuris	99	15.25
Hookworm and Ascaris	29	7.55
Ascaris and Trichuris	66	10.16
Triple infections	69	10.63

working with feces apt to harbor organisms of amoebic and bacillary dysentery and other pathogenic micro-organisms, and enabled us to delay examination of samples for several days whenever more pressing duties demanded our attention. The possibility of fraud and substitution in connection with samples of feces was taken into consideration, but in view of the interest that the students exhibited in this survey, the eagerness with which they awaited results of examinations, the numerous inquiries that they made concerning their individual cases and the requests for certification to the physician of the College of Agriculture for treatment, made it highly probable that each student submitted a sample of his own feces. It should also be stated in this connection that students at the Los Baños colleges live not only on the campus but also in adjoining settlements. Since students were required to submit samples of feces in alphabetical order, irrespective of classes

1. Only male students are in attendance at Los Baños. With few exceptions the ages range from twenty to thirty.

or other associations, the chances of practicing substitution were reduced to a minimum. It may be safely concluded, therefore, that each student submitted a sample of his own feces.

Each sample of feces was screened and the centrifuged sediment was usually examined in duplicate, and not infrequently in triplicate. Very few samples received but one examination. This procedure enabled us to detect very light infestations that would otherwise have been overlooked.

TABLE 2.—PREVIOUS RECORDS ON THE PREVALENCE OF INTESTINAL PARASITES IN THE PHILIPPINE ISLANDS

Number Examined	Locality	Percentage Infested	Percentage Infested with Hookworm	Percentage Infested with Ascaris	Percentage Infested with Whipworm	Authority
4,106*	Bilibid Prison	84.0	52.0	26.0	59.0	Garrison (1908)
385	Manila	89.0	13.45	53.22	87.60	Garrison and Llamas (1909)
1,000*	Taytay, Rizal	95.9	11.6	82.9	77.0	Garrison, Leynes and Llamas (1909)
1,087*	Bilibid Prison	70.0	16.7	37.5	40.0	Rissler and Gomez (1909)
6,108*	Las Piñas, Tuguegarao, Sta. Isabel, Ilagan, Isabela	89.83 74.13 86.28	11.14 8.01 46.38	77.21 73.55 60.59	53.4 25.9 6.23	Rissler and Gomez (1910)
119	Baguio	92.5	29.0	73.0	60.0	Chamberlain, Bloombergh and Kilbourne (1910)
183	Civil Hospital, Baguio	89.7	29.0	26.0	29.0	Same as above
932*	Cavite	85.6	2.4	67.2	65.1	Stitt (1911)
4,278	Cagayan Valley	85.46	54.37	62.04	7.99	Willets (1911)
150	....	.....	33.0	32.0	44.6	Tenney (1913)
500*	Manila (necropsies)	Over 90.0	16.6	41.2	34.4	Crowell and Hammack (1913)
400	Batanes Islands	100.0	25.5	92.8	46.7	Willets (1913)
1,000	General Hospital, Manila	84.8	18.7	46.5	60.6	Willets (1914)
7,843	Bilibid Prison	69.1	22.2	40.9	47.1	Willets (1914)
1,063*	Cebu Hospital	66.5	31.31	27.82	41.11	Garcia (1917)

* Protozoon findings included.

RESULTS OF EXAMINATIONS

The results of examinations are given below in tabular form (Table 1).

A comparison of our total number of positive cases with those recorded by other investigators shows that with few exceptions that appear to be due to local conditions, our percentage of infestations with roundworms corresponds to the prevalence of intestinal parasitism in the

native population so far as available data are concerned.[2] The records obtained by other investigators in the Philippines are shown below (Table 2).

It will be seen from Table 2 that in examinations which involved large numbers of persons the percentage of infestation is close to 85 in most cases, and is in agreement with our results. The higher percentages shown in Table 2 are probably due either to peculiar local conditions, or to examination of certain classes of persons that are exposed by habits and by occupation to infestation with roundworms.

INFESTATION WITH HOOKWORM

As shown in Table 1, of the 316 students that were found to harbor hookworms, 99 (15.25 per cent. of the total number examined) were infested with hookworm alone, a similar number showed a double infestation of hookworm and whipworm, while 49 (7.55 per cent.) were infested with hookworm and Ascaris. The number infested with hookworm, Ascaris and whipworm was 69 (10.63 per cent.).

A comparison of our findings with those of other investigators (Table 2) shows that our percentage is higher than most averages hitherto recorded for the Philippine Islands. The averages obtained by Garrison (1908) and by Willets (1911) are somewhat higher than our own average. The discrepancy is even greater than the figures indicate when it is remembered that Garrison and Willets based the results of their examinations on direct smears and doubtless missed very light infestations. It must be concluded that inmates of Bilibid Prison examined by Garrison in 1908 showed a considerably higher hookworm index than the normal population of the Philippines. In this connection a comparison of Garrison's findings (Garrison 1908) with reference to inmates of Bilibid Prison with those of Willets (1914), whose findings are based on examinations of prisoners on admission to Bilibid, shows that during the time that Garrison made his examinations conditions in Bilibid were apparently favorable to the spread of hookworm infections, otherwise the discrepancy in the results of the two investigators is difficult to explain.[3] The high percentage of hookworm infestation obtained by Willets (1911) appears to be due to the occupation of the persons examined, namely, field workers in tobacco plantations who go about their daily tasks barefooted. A similar explanation applies to the high percentage of hookworm infestations reported by Rissler and Gomez (1910) for Santa Isabel, Ilagan and Isabela.

2. Cestodes and trematodes are very rare in Filipinos and these parasites as well as protozoa are usually associated with nematodes.

3. Willets points out that improvement in conditions outside of the prison and the personal equation entering into the results do not account for the discrepancies.

Our own average would have been considerably lower if our examinations had not been based on concentrated fecal sediments and it is no exaggeration to state that about two thirds of our light infestations would have been overlooked in direct examinations of smears.

DEGREE OF HOOKWORM INFESTATION

Owing to the absence of hospital facilities on the university campus at Los Baños it was found almost impossible to secure specimens of hookworms passed following anthelminthic medication for the removal of worms, and no attempts were made to count the number of worms and to diagnose with accuracy the species of hookworms involved in this survey. The degree of infestation was judged by the scarcity or abundance of hookworm ova in fecal samples.

In a recent paper Smillie (1921) points out that while in individual cases the number of hookworm ova in stools frequently gives no trustworthy index to the number of parasites present, there exists nevertheless a definite relationship between the number of ova in stools and the number of hookworms harbored.

Only 47 positive hookworm samples showed an abundance of eggs that enabled us to make diagnosis readily. Of these only 9 samples contained eggs in great abundance, while 38 samples contained a moderate number of eggs, about one ovum in every four or five fields. The remaining 269 samples, approximately 85 per cent. of the hookworm positive samples, contained very few ova. In the latter samples from 1 to 3 eggs were generally found in each preparation and in many cases two or more slides were examined before a single egg was found. These data appear to indicate that while approximately 50 per cent. of those examined harbored hookworms, the vast majority of those that were infested were apparently lightly parasitized, as far as could be judged by the scarcity of ova in fecal sediments.

These observations on the lightness of infestation of Filipinos with hookworm are in harmony with those of other investigators. Garrison (1908) found that while 52 per cent. of the inmates of Bilibid Prison were infected with hookworm, clinical manifestations of the disease were not in evidence. Garrison makes no statement as to the degree of infestation with hookworm, but inasmuch as the clinical manifestations of hookworm infection are generally an index to the number of parasites present, it is very probable that Garrison's cases were, on the whole, lightly infested.

Garrison, Leynes and Llamas (1909) report that in the course of their parasitological survey of one thousand inhabitants in the town of Taytay they found very few hookworm ova in cover glass preparations, and that the greatest number of worms that they recovered after

treatment was 14. Rissler and Gomez (1910) confirm the lightness of hookworm infestation in natives of the Philippine Islands. These investigators state that in most cases that came under their observations from 5 to 10 hookworms were expelled after anthelminthic treatment and that in many cases only 2 or 3 parasites were recovered after treatment.

Willets (1911) found in over 4,000 examinations 54.37 per cent. positive for hookworm, but the degree of infestation was extremely light. He states: "In my opinion not less than 95 per cent. of the hookworm infections seen were distinctly mild in degree." Gomez (1911), in a study of 26 selected cases of hookworm infections, recovered from 1 to 20 specimens following anthelminthic treatment, having previously found few eggs in fecal samples. Walker, Guzman and Concepcion (1914) examined 58 persons in connection with a sanitary survey at Mindoro and found that 48.27 per cent. were infected with hookworms. Musgrave (1914) in collaboration with several physicians studied these cases clinically and found that most of these infections were light in character and that the clinical inefficiency of these patients was but little, if at all, lower than the general average. Haughwout and Horrilleno (1920) admit that hookworm infestations in the Philippines are frequently very light and without clinical manifestations, but they state that a number of cases of severe hookworm anemia have come to their attention.

SPECIES OF HOOKWORM IN THE PHILIPPINES

Numerous measurements of hookworm ova were made with a view of determining the species involved. By far the vast majority of eggs measured ranged from 68 microns to 75 microns in length, and were probably those of *Necator americanus,* while smaller eggs, 55 microns to 60 microns in length, probably those of *Ancylostoma duodenale,* were not uncommon. Since no accurate determination of hookworm species can be based on egg measurements, because the size of eggs of the two species of hookworms commonly parasitic in man doubtlessly overlap, no definite conclusions as to the relative abundance of Ancylostoma and Necator in the Philippines can be drawn from our data.

SIGNIFICANCE OF HOOKWORM INFECTIONS IN THE PHILIPPINES

Most investigators who have studied medical zoological problems in the Philippines are of the opinion, based upon considerable laboratory investigations and clinical findings, that hookworm infestation produces apparently no ill effects on Filipinos. The statements of Garrison (1908) concerning the absence of clinical symptoms in Filipinos harboring hookworms are confirmed by other investigators, notably by Willets, Gomez and Garcia. So far as the medical records of our

cases are concerned, including those with moderate to heavy infestations, there appears nothing to indicate effects of hookworm disease in the strict sense.

Garrison, Leynes and Llamas (1909) examined the hemoglobin content of hookworm positive cases but failed to correlate a low hemoglobin content with hookworm infection, even in cases of heavy infestations. Gomez (1911) compared the percentage of hemoglobin and the number of red and white blood corpuscles of 26 Filipinos infected with hookworm with those of 10 non-infected Filipinos and failed to find any noteworthy differences between the two groups. He concluded that the absence of clinical symptoms in Filipinos harboring hookworms is not due to racial immunity but to the small number of infecting organisms. Gomez has overlooked the fact that light infestations may, in themselves, indicate an immunity. Unless it can be shown that climatic conditions or conditions of the soil or other physical factors in the Philippines are inimical to the development of hookworm eggs and the survival of the larvae in the soil, the view that Filipinos have a racial immunity to hookworms is tenable. Gomez has also overlooked the fact that marked cases of hookworm anemia have been reported by certain investigators in cases in which few parasites were involved, whereas in Filipinos, so far as available information is concerned, even moderate and heavy infestations are often unaccompanied by anemia.

In view of the generally recognized importance of hookworm infection it is highly important that further investigations should be carried out before the lack of medical significance of hookworm infections in the Philippines is accepted as conclusive. Epidemiological investigations should be supplemented by experimental studies on the development of hookworm eggs and larvae in the soil, and on the duration of life of infective larvae in Philippine soils. Effects of tropical sunlight and of flooding rains on the ova and larvae of hookworms require thorough investigation.

INFESTATION WITH WHIPWORM

As shown in Table 1 of the 357 (55 per cent.) positive whipworm samples, 123 (18.95 per cent.) were infested with Trichuris alone. Double infections of Trichuris and hookworm numbered 99 (15.25 per cent.), double infections of Trichuris and Ascaris numbered 66 (10.16 per cent.), while triple infections of Trichuris, Ascaris and hookworm numbered 69 (10.63 per cent.). Of the 386 cases of whipworm infestation only 55 ranged from moderate to heavy and in these only four samples showed an abundance of eggs in feces. In the remaining 302 cases eggs were very scarce in fecal sediments.

Record obtained by other investigators on the prevalence of whipworm infestations in the Philippine Islands show considerable variation,

depending to a certain extent on geographical locations. Our average is in agreement with the general averages obtained in examinations of prisoners at Bilibid, but the fact that all investigations that are summarized in Table 2 are based on direct smears, whereas our records are based on concentrated sediments, must be taken into consideration. That Trichuris infestations may be accompanied by a severe progressive anemia has been recognized ever since Askanazy (1896) reported the presence of hemoglobin products in this parasite. The blood-sucking habits of Trichuris have been fully emphasized by Guiart (1907) and by others, and heavy infestations with these organisms are generally coming to be recognized as of considerable medical importance.

INFESTATION WITH ASCARIS

About one out of three students examined was infested with Ascaris, the total number infested being 229 (35.28 per cent.). As shown in Table 1 pure Ascaris infestations numbered 45 (6.93 per cent.), double infestations of Ascaris and hookworm numbered 49 (7.55 per cent.), double infestations of Ascaris and whipworm numbered 66 (10.16 per cent.), while triple infestations involving Ascaris numbered 69 (10.63 per cent.).

The majority of Ascaris positive samples, namely, 139 showed an abundance of eggs, whereas 90 samples showed comparatively few eggs. Among the latter many showed a pure culture of so-called abnormal Ascaris eggs that are commonly considered to be unfertilized eggs and are probably indicative of infestation with one or more female worms without the presence of male worms.

So far as concerns Ascaris infestation, our average is considerably lower than that recorded by most investigators, but is in general agreement with the averages found in Bilibid. As in our own cases, the inmates of Bilibid are nearly all adults. It has been repeatedly shown in helminthological surveys that children are more heavily infested with Ascaris than adults, the degree of infestation decreasing within certain limits with advancing age. Our average represents, therefore, the average for adult Filipinos.

The pathogenic rôle of Ascaris has been so well established by recent investigations on the migrations of the larvae through various organs, including the lungs, that no further emphasis is necessary to incriminate Ascaris as a probable important factor in respiratory diseases of children. Whether an invasion of the lungs by Ascaris larvae breaks down the vitality of these organs and increases their susceptibility to infection with tubercle bacilli and other pathogenic organisms is a question that still awaits solution.

SUMMARY AND CONCLUSIONS

The majority of normal adult Filipinos (approximately 85 per cent.) are infested with one or more intestinal roundworms.

Approximately 50 per cent. are infested with hookworm, but the vast majority so lightly parasitized, as judged by the scarcity of ova in feces, that direct smears may fail to reveal evidence of infestation.

Both species of hookworm commonly parasitic in man apparently occur in the native population of the Philippines.

Filipinos appear to be resistant to hookworm infection and do not appear to show, as a general rule, clinical symptoms of hookworm disease.

Whether a racial immunity is involved in this resistance or whether purely physical and other conditions unfavorable to the life of hookworm eggs and larvae in the soil are responsible for the light infections still remains to be determined.

Ascaris and whipworm are commonly associated with hookworm, the degree and incidence of Trichuris infection corresponding to those of hookworm infection. While Ascaris appears to be less common in adults than the above mentioned species it is generally present in greater numbers than the other species of intestinal nematodes mentioned in this paper.

The prevailing notions concerning the lightness of hookworm infestation and the absence of clinical symptoms in Filipinos harboring these parasites, though supported by considerable amount of data, should be confirmed by further investigations before the lack of medical importance that is now attributed to hookworm infections in the native population is accepted as an established fact.

LITERATURE CITED

Askanazy, M. 1896.—Der Peitschenwurm ein blutsaugender Parasit. Deutsch. Arch. klin. Med., 57: 104-117.

Chamberlain, W. P., Bloombergh, H. D., and Kilbourne, E. D. 1910.—Examinations of Stools and Blood Among Igorrots at Baguio, Philippine Islands. Phil. Jour. Sci., 5 (B): 505.

Cole, E. L. 1907.—*Necator americanus* in Natives of the Philippine Islands. Phil. Jour. Sci., 2(B): 333.

Crowell, B. C., and Hammack, R. W. 1913.—Intestinal Parasites Encountered in Five Hundred Autopsies, with Report of Cases. Phil. Jour. Sci., 8(B): 157.

Garcia, F. 1917.—Common Intestinal Parasites. Phil. Jour. Sci., 12(B): 25.

Garrison, P. E. 1908.—The Prevalence and Distribution of the Animal Parasites of Man in the Philippine Islands, with a Consideration of Their Probable Influence upon the Public Health. Phil. Jour. Sci., 3(B): 191.

Garrison, P. E., and Llamas, R. 1909.—The Intestinal Worms of 385 Filipino Women and Children in Manila. Phil. Jour. Sci., 4(B): 185.

Garrison, P. E., Leynes, R., and Llamas, R. 1909.—Medical Survey of the Town of Taytay. X. Animal Parasites of the Intestines. Phil. Jour. Sci., 4(B): 257.

Gomez, L. 1911.—A Clinical Study of the Hookworm Infection in the Philippines. Phil. Jour. Sci., 6(B) : 239.

Guiart, J. 1907.—Le trichocephale vit aussi dans l'intestin grele et se nourrrit de sang. Bull. Soc. med. Hop., Lyon, 7 : 37.

Haughwout, F. G., and Horilleno, F. S. 1920.—The Intestinal Parasites Found in One Hundred Sick Filipino Children. Phil. Jour. Sci., 16 : 1.

Musgrave, W. E., Vasquez, Gutierrez B., and Cox, S. L. 1914.—Sanitary Survey of the San Jose Estate and Adjacent Properties of Mindoro Island, etc. 6. The Disease Index (B) Clinical Examinations. Phil. Jour. Sci., 9(B) : 173.

Rissler, R. S., and Gomez, L. 1909.—Intestinal Parasites Prevalent in the Philippines and Their Effect on the Health of Individuals and of the Community. Memorias Premiadas en el Concurso Medico abierto en Manila con ocasion de las Fiestas Jubilares del Ateneo de Manila en Diciembre, 1909; pp. 85-132.

Rissler, R. S., and Gomez, L. 1910.—The Prevalence of Intestinal Parasites in Rizal and Cavite Provinces and in Cagayan Valley. Phil. Jour. Sci., 5(B) : 267.

Schwartz, B., and Tubangui, M. A. 1922.—Uncommon Intestinal Parasites of Man in the Philippine Islands. *In press.* (Phil. Jour. Sci., 20.)

Smillie, W. G. 1921.—A Comparison of the Number of Hookworm Ova in the Stool with the Actual Number of Hookworms Harbored by the Individual. Amer. Jour. Trop. Med., 1 : 389.

Stitt, E. R. 1911.—A Study of the Intestinal Parasites Found in Cavite Province. Phil. Jour. Sci., 6(B) : 211.

Tenney, E. S. 1913-14.—Some observations on the prevalence of Intestinal Parasites in the Philippine Islands. Amer. Jour. Trop. Dis., 6 : 44.

Walker, E. L., Cuzman, A. R., and Concepcion, I. 1914.—Sanitary Survey of the San Jose Estate and Adjacent Properties of Mondoro Island, Philippine Islands, with Especial Reference to the Epidemiology of Malaria. 6. The Disease Index. (A) Laboratory Examinations. Phil. Jour. Sci., 9(B) : 167.

Willets, D. G. 1911.—A Statistical Study of Intestinal Parasites in Tobacco Haciendas of the Cagayan Valley, Philippine Islands. Phil. Jour. Sci., 6(B) : 77.

1913.—General Conditions Affecting the Public Health and Diseases Prevalent in the Batanes Islands, P. I. Phil. Jour. Sci., 8(B) : 49.

1914.—Intestinal Parasitism, Particularly Entamoebiasis, in Patients of the Philippine General Hospital, Manila, P. I. Phil. Jour. Sci., 9(B) : 81.

1914.—Intestinal Helminthiasis in the Philippine Islands as Indicated by Examinations of Prisoners upon Admission to Bilibid Prison, Manila, P. I. Phil. Jour. Sci., 9(B) : 233.

TRYPANOSOMA BRUCEI AS A FILTERABLE VIRUS—A PRELIMINARY NOTE

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The satisfactory study of a filterable virus presents many technical difficulties. The danger of drawing sweeping conclusions from improperly controlled experimental conditions is often more real than at first may be apparent. The lack of uniformity in the procedure of various investigators, together with the difficulty of establishing a type experiment have been pointed out and discussed by Meyer (1914). Much of the confusion exhibited in the results of various workers is directly attributable to the failure of duplicating closely the experimental conditions involved. Recognition of this lack of uniformity in filtration experiments and a plea for the standardization of methods was made early by Marchoux (1908). Such standardization of apparatus and technic is absolutely essential if reported findings are to be of any comparative value. The same writer calls attention to the various physical factors affecting both the filter and the material to be filtered, any one or all of which may operate to modify the results greatly. Because of these limitations, and because of the fact that standardization has not yet progressed to that point wherein results may be strictly interpreted on a comparative basis, repetition of most if not all of the earlier work using a uniform technique would seem to be highly desirable. The controversies between Remlinger, Celli and Dublasi and others concerning the virus of rabies; or of Nicolle and Adil-Bey and Todd on the virus of cattle plague, may be cited as cases in point. The type of bougie, filtration temperature and pressure, duration of filtration, dilution of the material to be filtered, albumen content of such material, as well as the nature of the diluent itself, are some of the most important factors upon which much depends.

The conception that all filterable viruses are of an invisible or 'utramicroscopic' nature has long since been abandoned. The observations of Borrel (1908) and Bosc (1902) on sheep-pox; Novy's claim regarding the filtration of *T. lewisi* (1904), Wolbach and Binger's (1914) work on *Spirochaeta elusa;* Novy and Knapp's (1906) studies on *Spirillum obermeieri;* as well as the well-known works of Guarnieri, Calkins and others on the peculiar inclusion bodies found in vaccinia, trachoma and hydrophobia, represent some of the attempts to demonstrate microscopically the filterable bodies. In those cases which have not yet yielded positive results, a further acquaintance with the life

cycle involved may possibly enable us to demonstrate a visible entity during certain stages in the development of many of the so-called 'ultramicroscopic viruses.' Of interest in this connection is the recent work of Dios and Oyarzabal on the trypanosomes of surra and of mal de caderas. These authors describe an intracorpuscular form of the specific trypanosome concerned in the blood of horses experimentally infected. Kraus, Dios and Oyarzabal claim also that there exists an invisible stage in the life cycle of certain of the bovine piroplasmas and trypanosomes. They offer these observations in support of Schaudinn's generalization that there is an invisible stage in the life cycle of some protozoa. In a recent publication Kleine criticizes these findings and attributes the "invisible stage" to a lack of careful search of the infectious material. He asserts that with proper technique and diligence the parasites may be demonstrated. Schepilewsky's claim to have seen thread-like appendages on trypanosomes by the use of dark-field illumination is also worthy of note.

With these sketchy notes pertinent to some of the problems involved, the various channels of error, and the somewhat chaotic state of our knowledge of the filterable viruses as a group, a few words may now be said relative to observations which deal more directly with the nature of the present investigation. Novy and Knapp (1906) observed that *T. lewisi* in culture underwent various morphological changes. The minuteness of some of these forms led them to experiment with filtrates of such culture material but exact details of their procedure are not available. The material was diluted with salt solution and filtered through Berkefeld candles at a pressure of five pounds. A perfectly clear filtrate was obtained which upon injection into white rats yielded three positive infections in nine attempts. Similar experiments with cultures of *T. brucei* and with suspensions of the blood and organs of infected animals yielded negative results. Wolbach, Chapman and Stevens (1915) in discussing this work state that "these experiments were done under high pressure (50 lbs. plus) with Berkefeld filters which had been reduced by sandpapering." Reference to the article by Novy and Knapp quoted above conveys the impression that such conditions obtained in the experiments with *Spirillum obermeieri* but not in the work with trypanosomes. In a personal communication, Dr. Novy refers to the importance of using cold liquids and of speed both in filtration and injection of filtrates, but mentions nothing regarding the other details. Bruce and Bateman (1908) working with both cultures and organ suspensions of animals infected with *T. brucei* or *T. evansi* were unsuccessful in their attempts to produce infection with the filtrates. They passed the infectious material through Berkefeld filters controlled with *Micrococcus melitensis* and concluded that neither trypanosome produces, in the bodies of animals or in culture, forms

that can pass through the pores of a bacteria-proof filter. Bruce and his associates (1911) working with filtrates of the intestinal tract of flies known to be infected with *T. gambiense* obtained entirely negative results. Wolbach, Chapman, and Stevens (1915) stimulated by the results of Novy and MacNeal's work with *Spirochaeta duttoni* and the confirmation of these results by Todd and Wolbach (1914), repeated their earlier experiments, using *T. brucei, T. lewisi* and *T. gambiense*. The filtration was accomplished with Berkefeld "V" filters, using pressures ranging from gravity to 50 pounds, and the results were controlled with a suspension of *B. prodigiosus* or of *Staphylococcus citreus*. Twenty-four experiments were performed, and in the case of every bacteriologically sterile filtrate, inoculation into white rats failed to infect. The control rats which had been inoculated with the unfiltered material developed infection. These writers attribute the positive results of Novy and MacNeal to the thinning down of the filters used by them and do not believe that these shaved filters would have yielded bacteria-free filtrates under the conditions of their own experiments. They state further that even in actively growing cultures they have not seen forms whose least dimension did not exceed the diameters of the bacterium used for control. In view of their results, they conclude therefore that trypanosomes from cultures and from animal tissues are not filterable through bacteria-proof filters.

Experimental Work

On January 27, 1921, through the courtesy of Dr. F. G. Novy of Ann Arbor, we received two cultures each of *T. lewisi* and *T. brucei*. These cultures were on rat's blood agar. Of six white rats injected intraperitoneally with saline suspensions of the cultures of the latter, one developed infection. From this source, a series of white rats and guinea-pigs have been inoculated and the strain maintained for study. It may be of interest to remark that dark field examination of the original culture material failed to reveal any living forms, and doubt was entertained regarding the viability of the material.

In the course of the examination of fresh and stained material derived from the blood and organs of animals infected with *T. brucei*, preparations were secured revealing curious transformations of the parasites (1921). Granular nuclear detritus; minute ovoid or round Leishmania-like forms; cycle-shaped Herpetomonad and Crithidial bodies (both flagellated and non-flagellated); and typical trypanosomes with all of the characteristics of the genus—these and other appearances suggestive of intermediate stages were often found not only in the same preparation but often in the same field. It is the writers' opinion that these bodies are not to be confused with the degeneration changes

described by Laveran and Mesnil as occurring when trypanosomes are allowed to come into contact with serum, saline and other deleterious substances. They may or may not be related to the 'latent bodies' of Moore and Breinl or to the schizogenous forms described by Walker in the spleen of animals infected with *T. evansi*. It was at first thought that these findings were exceptional. It has since been found possible to demonstrate them on numerous occasions in the liver, spleen and occasionally the heart's blood of animals at or shortly after death. A search of the organs before death had supervened has not yet been undertaken. It is reasonable to presume however that such morphological changes from supposed type will be encountered to some degree at least at some appreciable time before the death of the animal.

The routine procedure in the filtration experiments has been as follows: The material used came from animals handled at or as closely after death as possible. The animal was secured on a small dissecting board and the thoracic and abdominal viscera were exposed with aseptic precautions. This care was exercised since contaminating organisms naturally would confuse the interpretation of the bacterial control. The heart was removed in toto and placed immediately in a sterile mortar containing 30 to 40 cc. of sterile citrated saline (10% sodium citrate in physiological saline). The blood in the thoracic cavity was aspirated with a sterile Luer syringe without needle. Portions of the lungs, liver, spleen, kidneys, inguinal lymph nodes and bone marrow were also removed and placed immediately in the same saline. In each case, smears of the various organs were made and later stained by the writers' modification of Wright's method (1921). The material in the saline was then minced carefully with a pair of sterile scissors and then ground further with a sterile pestle. To the mixture was then added a loopful of a 24-48 hour slant culture of *B. prodigiosus*. After mixing, the maceration was filtered through one or two thicknesses of sterile gauze directly into the aluminum receptacle enclosing the filter candle. This procedure was carried out immediately after death with at least half of the series. In one successful instance however, approximately 36 hours had elapsed before autopsy.

With Mandler filters of diatomaceous earth, the filtration was accomplished by means of a suction pump using pressures varying between twenty and twenty-five pounds and with time from twenty-five to forty-five minutes in different experiments. Preliminary examination had been made of all animals in order to be assured that no trypanosomes of any sort were present before using for experimental purposes. Moreover these animals had been in stock for many weeks before use. The filtration usually yielded approximately 3 cc. of colorless or straw-tinted liquid. One or two guinea-pigs were inoculated intraperitoneally with 1.5-2 cc. of the filtrate. A control animal was inoculated with the

same quantity of the unfiltered material. Immediately following this process, three 2 mm. loopfuls of the filtrate were placed on glucose agar to determine the absence of *B. prodigiosus* while at the same time one loopful of the original maceration was placed on another slant. The purpose of this technique was of course to furnish proof that the candle was of grain sufficiently fine to intercept the bacterial micro-organism. Such tubes were then incubated at 37 C. for 24 hours, followed by room temperature for one week to insure maximum production of pigment.

To date twenty-five experiments have been performed. In four of this series, the filters proved defective by bacteriological control. In two other cases, the control animals failed to develop infection. In the nineteen remaining experiments wherein the control animals developed positively and in which bacteriological cultures of the filtrate remained negative for growth, ten of the animals inoculated with bacteria-free filtrates developed infection of *T. brucei.*

Discussion and Results

The possibility of the existence of forms still smaller than those described in the stained smears, and their relation or identity with the filter-passing entity, must be borne in mind as a distinct possibility. The divergence in the results reported by various investigators may be due to (1) differences in the technique, (2) peculiarity of the particular strain, and (3) to the presence or absence of the specific form changes alluded to. In the opinion of the writers, the last condition should prove to be an important postulate.

1. Form changes are described in the blood and organs of white rats and guinea-pigs dying of infection with *Trypanosoma brucci.*

2. The viability of these forms is evidenced cytologically by the mitotic figures and physiologically by their infectiveness.

3. The blood and organs of guinea-pigs dying or dead of infection with *T. brucci,* and in which the form changes described occur, when filtered through a bacteria-proof filter, yield filtrates which in ten cases out of nineteen were infective for guinea-pigs.

4. The possible relation between this filterable virus and certain of the described 'involution forms' or with a problematical ultramicroscopic stage in the life of this parasite is pointed out.

References Cited

Borrel, A. 1903.—Etude Expérimentale de la Clavelée. Ann. Inst. Pasteur, 17:123.

Bosc, F. J. 1902.—De l'Existence dans toutes les Lesions Claveleuses Virulentes et dans le Sang de Corps Particuliers de Structure Précise, etc. C. R. Soc. Biol., 54:11.

Bruce, D., and Bateman, H. R. 1908.—Have Trypanosomes an Ultramicroscopic Stage in their Life-History? Proc. Royal Soc. London, 80:394.

Bruce, D., Hamerton, E. D., Bateman, H. R., et al. 1911.—Eleventh Report of the Sleeping Sickness Commission of the Royal Society of London, 3:437.

Marchoux, E. 1908.—Bougies Filtrants et Virus Invisibles. C. R. Soc. Biol., 65:82.

Meyer, K. F. 1914.—Filterable Viruses. Amer. Vet. Rev., Nov.-Dec.

Novy, F. G., and MacNeal, W. J. 1904.—On the Filtration of Trypanosomes. Trans. Mich. Acad. Sci., 6:1 (Personal Communication).

Novy, F. G., and Knapp, R. E. 1906.—Studies on Spirillum Obermeieiri and Related Organisms. Jour. Inf. Dis., 3:291.

Reich, W. W. 1921.—Some Curious Morphological Experimental Variations Found with Trypanosoma Brucei. Thesis M. A., Univ. Cal.

Todd, J. L., and Wolbach, S. B. 1914.—Concerning the Filterability of Spirochaeta duttoni. Jour. Med. Res., 30:27.

Wolbach, S. B., and Binger, C. A. L. 1914.—The Cultivation of a Free-Living Filterable Spirochaete (*S. elusa*, n. sp.). Jour. Med. Res., 30:9.

Wolbach, S. B., Chapman, W. H., and Stevens, H. W. 1915.—Concerning the Filterability of Trypanosomes. Jour. Med. Res., 33:107.

NOTES ON THE LARVAE OF *MONILIFORMIS MONILIFORMIS* (BREMS.) FOUND IN AFRICAN COCKROACHES

T. Southwell

The material on which these notes are based consisted of about 30 cysts which were collected by Dr. J. W. S. Macfie of Accra, Gold Coast, West Africa, from the abdominal cavities of 2 cockroaches (*Periplaneta americana*). Among these cysts were found 8 or 9 gravid female specimens of *Oxyuris blattae-orientalis* Hammersch., 1847, recognized by the esophagus possessing a lateral pouch.

Travassos (1917) splits up the family *Gigantorhynchidae,* Hamann, 1892, into 2 sub-families, viz., *Gigantorhynchinae,* Travassos, 1915, and *Prosthenorchinae,* Travassos, 1915. The sub-family *Gigantorhynchinae* contains 5 genera, 1 of which is *Moniliformis,* characterized by the possession of numerous small hooks. There are 2 species in the genus, viz., *M. moniliformis* and *M. cestodiformis* (v. Linstow, 1904)

MONILIFORMIS MONILIFORMIS (BREMS.) TRAVASSOS, 1915

Syn.—*Echinorhynchus moniliformis* Bremser, 1819
Gigantorhynchus moniliformis (Brems.) Railliet, 1893
Echinorhynchus grassii Railliet, 1893

The adult form of *M. moniliformis* has been recorded from the following hosts: *Man, Sciurus niger, Eliomys quercinus, Cricetus cricetus, Mus rattus, Mus norvegicus, Mus albipes, Microtus arvalis, Canis familiaris, Lepus sinditicus* and *Erinaceus algirus.* The larva occurs in *Periplaneta americana* and *Blaps mucronata.* It has also been recorded from *Putorius putorius* and *Circus pygargus,* but it is extremely rare in the 2 latter hosts. Both the larva and the adult are of world wide distribution.

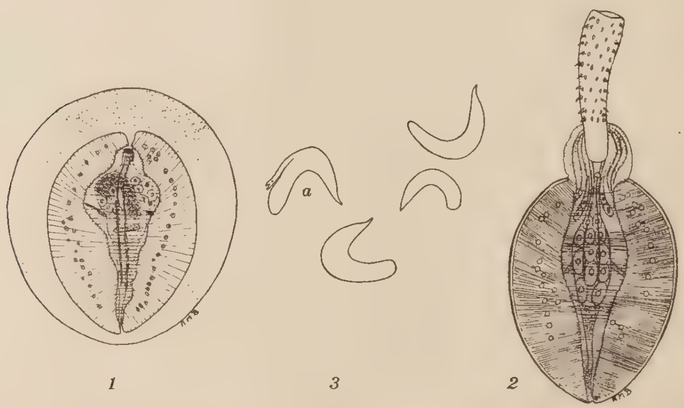
The larva found in the cockroach is enclosed in an extremely delicate cyst which is easily lost. In shape it is globular; its diameter varies from 0.9 to 1.2 mm. In young larval forms it is partly filled with granular material which gradually disappears as the larva becomes older (Text fig. 1).

The larva lies within the cyst wall and is milky white in appearance. In shape it resembles a split pea, except that it is more heart-shaped, and has one surface slightly concave whilst the other (convex) surface has, in addition, a broad ridge running across it antero-posteriorly. The size of 7 of these larvae varied as follows:

No.	Length, micra	Breadth, micra
1	800	580
2	910	740
3	984	750
4	1060	760
5	1060	800
6	1100	770
7	1120	810

On microscopic examination the larval body-wall presents a striated appearance, and a number of giant nuclei lie in small annular lacunae placed centrally in the body wall (Text figs. 1 and 2). The body cavity in which the rudiments of the reproductive system lie narrows behind and opens to the exterior posteriorly.

The proboscis was extruded in one specimen only, and in this case its total length was 620μ and its breadth 155μ. The posterior third of



Text figures. *Moniliformis moniliformis,* larva. *1,* Cyst from cockroach, × 35. *2,* Larva from cyst, × 35. *3,* Hooks from proboscis of larva; *a,* hook with rough posterior end; × about 300.

the proboscis (200μ in length) was unarmed, the anterior two thirds (400μ in length) being armed. There are about 12 longitudinal rows of hooks and about 6 or 7 hooks in each transverse row. The hooks near the anterior extremity are much larger than the posterior hooks and there is considerable variation in the shape of both types (Text fig. 3). They are all roughly semicircular, having one extremity sharply pointed and the other extremity either bluntly pointed or even truncated. The outer face of the hook near the blunt extremity frequently presents a jagged appearance (Text fig. 3a). The measurements of the largest and smallest hooks seen are as follows:

	Greatest breadth (diameter)	Greatest height (radius)
Large hook	32 micra	36 micra
Small hook	21 micra	17 micra

The proboscis receptacle is bulbular, measuring 375μ in length and 270μ in breadth. The brain is situated in the bulb just behind the proboscis (Text fig. 2). A number of stout muscle fibers arise at the junction of the armed and unarmed parts of the proboscis and become attached posteriorly to the anterior internal body-wall.

In the specimen examined, which was a female, the lemnisci are small, rudimentary and club-shaped. The suspensory ligaments can be traced with difficulty in whole mounts under the oil immersion; they run through the body cavity almost to the posterior extremity. About the middle of the body cavity these ligaments bear a mass of about 12 cells, densely crowded together, these being the rudiments of the female genitalia; they are attached to the wall of the body cavity by a pair of muscles, one on each side, running almost at right angles to the length of the body cavity. In those specimens in which the proboscis was retracted the latter organ occupied the greater part of the body cavity and the mass of cells referred to above were divided into two groups one on each side.

The larva, on being swallowed by a suitable host, escapes from its cyst (if this has not already been lost) and develops directly into the adult. Infections in man are not common and the way in which infection takes place is a little obscure. The larvae, occurring as they do in the abdominal cavities of cockroaches and beetles, are not passed in the feces, and infection from this source is therefore impossible. Infection in man depends on (1) infected cockroaches or beetles being swallowed whole, or (2) pollution of food by cysts from disintegrated cockroaches or beetles.

References Cited

Chandler, A. C. 1921.—Notes on the Occurrence of Monilformis sp. in Rats in Texas. Jour. Parasit., 7: 179-183.

Hamann, O. 1891.—Die Nemathelminthen. Monogrophie der Acanthocephalen (Echinorhynchas). Jena; 119 pp., 10 pl.

Travassos, L. 1917.—Contribuções para o conhecimento da fauna helmintologica braziliera. Mem. Inst. Oswaldo Cruz, 9: 1-62; 25 pl.

Ward, H. B. 1917.—"Echinorhynchus moniliformis" in North America. Jour. Parasit., 3: 141.

SOME NEW METHODS IN NEMATODE TECHNIQUE *

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The following article is a digest of some methods for the handling of nematodes, which the author has worked out and found to be rapid and reliable, eliminating considerably the difficulties encountered in dealing with these most refractory organisms.

The material freshly collected and freed from adhering dirt and slime is placed in the following killing, clearing and dehydrating fluid, for convenience designated as "Carnoy-phenol":

Absolute alcohol	20 c.c.
Chloroform	15 c.c.
Glacial acetic acid	5 c.c.
Phenol crystals to raise the volume by	10 c.c.
Total	50 c.c.

As this mixture is water free and very corrosive, it must be stored in glass stoppered bottles until used and also should not be kept more than 2 weeks as an ester formation decreases its efficiency. In preparing specimens, it is most easily handled in Syracuse crystals, the edges of which have been dipped in hot paraffin to prevent the liquid from creeping over the rims and furthermore operations in these dishes may be easily controlled under a binocular or a compound microscope.

If material is killed in Carnoy-phenol, only 2 operations are required to bring the objects into paraffin or balsam, and one to clear them in glycerine; if killed in other media and stored in alcohol, 3 operations will attain the same end. Nematodes may be taken from 70 to 80 per cent. alcohol, glycerine, lacto-phenol, or formol in which they have been stored and placed directly in the fluid. Smaller worms are cleared almost instantly so that a rapid survey if desired may be made of their internal organization after which they may be removed to 95 or 80 per cent. alcohol for storage. If the larger specimens do not clear sufficiently at first, almost any degree of clearing may subsequently be obtained by allowing the fluid in which the worms are placed to evaporate, the degree of clearing being proportional to the amount of evaporation. Still greater transparency is obtained by adding glacial acetic acid and phenol to the worms.

With specimens now in the fluid they may be prepared either for sectioning or for mounting in balsam with equal ease. Oils of synthetic wintergreen and oleum cidri ligni (Merk) or chloroform may be dropped *slowly* into the dish with the specimens and mixed thoroughly

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* Contributions from the Zoological Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 209.

by agitation. The change must be very gradual at first, when the tissues are hardening but as soon as the mixture is three quarters clearing fluid, the greater part may be drawn off and the pure liquid added more rapidly and allowed to remain for 10 to 15 minutes or longer. If now infiltration by paraffin is desired the wax is shaved into the dish with the specimens in clearing fluid and the whole set aside in a slightly warm place for 2 hours when the worms may be placed in pure paraffin, melting at 58 C. and after proper infiltration imbedded. Most stains follow Carnoy-phenol admirably.

To mount whole preparations in balsam the procedure is the same, including the bringing of the worms into clearing fluid; wintergreen is here to be preferred to the other clearing fluids in general laboratory use because of its rapid penetrating power; xylol shrinks tissues too readily and should be entirely avoided. Now the Syracuse crystal bearing the worms in a small quantity of oil is tipped only slightly and a large drop of pure, unthinned, paper-filtered Canada balsam, is placed on the sloping bottom of the dish away from the worms and the whole covered. The resin will flow slowly down and diffuse throughout the oil and specimens in the course of 2 or 3 hours. Should the resulting resinous mixture be too thin to dry rapidly upon mounting the objects, more balsam may be added as before. It is important not to rush this process because the thinner medium within the worms will move through to the exterior faster than the balsam can penetrate to the interior with the result that the pressure becomes less within than without and unless the cuticula is thick, collapsing will result; but in all cases the more volatile fluids will vaporize under this reduced pressure and fill the body cavity and interstices between the organs with gas so that the preparations are again valueless, being utterly opaque. If collapsing has not taken place, the difficulty may be remedied by thinning the balsam with chloroform or benzol until the bubbles are gone, then controlling evaporation until the thickness of the fluid is again suitable for mounting. However, should collapsing as well have occurred, and should the specimens be valuable enough to warrant saving, restoration may be accomplished by running the worms back to Carnoy-phenol and leaving them there until the collapsed portions have plumped out. If this does not occur spontaneously, a slight manipulation by rolling the worm gently will usually restore shape. Where the specimens are large, it is desirable to puncture the cuticula in several places before balsam clearing, taking care not to injure the underlying organs.

Except for low power work with a microscope, toto staining is of little value in examination of relatively large specimens because of the marked tendency it has to mask the finer details of structure which one is desirous of seeing when using higher magnifications. Much greater latitude for observation is better obtained by utilizing degrees of clear-

ing rather than staining. Permanent mounts may be made of glycerine-prepared specimens in glycerine jelly properly sealed against evaporation, or material may be mounted after suitable preparation either in camsal-balsam, cedar immersion oil, or Canada balsam. These 4 mounting media will give a differential clearing indicated by the following approximate indices of refraction: 1.476, 1.47, 1.520, and 1.535 (Lee 1913), respectively. To prepare the specimens for passage into these media, they are first placed in Carnoy-phenol and then brought into the clearing fluids most suitable for passage into the mounting medium. For glycerine jelly mounts, the phenol reagent is replaced by pure glycerine; camsal-balsam is preceded by clearing the material in camsal, a liquid formed by the mutual solution of salol (phenyl-salicylate) and gum camphor; immersion oil follows thin cedar oil; and Canada balsam replaces oil of wintergreen. The process of clearing is accomplished as previously explained.

Another excellent medium for small, very transparent worms, is "Diaphane" a resinous medium employing gum Sandarac on the order of Gilson's "Euparal" which, because of its low index of refraction shows greater detail in the cleared specimens than balsam. The nematodes are cleared carefully from the phenol reagent by camsal and allowed to harden for a short time in this fluid. Then they may be transferred to diaphane, diluted to about one half strength by absolute iso-butyl alcohol, and allowed to clear by gentle evaporation of the alcohol. A slightly greater degree of clearing may be obtained by preparing the worms in the same manner and transferring to pure Canada balsam with 5 to 10 per cent. camsal well diluted with iso-butyl alcohol. This, too, makes an admirable, but very slow drying, tough, elastic medium.

It sometimes happens that specimens will accidentally become dried out through breakage of the glass container or neglect to stopper it properly. Such objects may be very well and often completely restored by the following method, such that staining, sectioning or toto-mounting may be undertaken. The dried worms are soaked in 35 to 50 per cent. alcohol to which an equal volume of lactic acid is added. If they do not soften and plump out to normal size, more acid may be added. When restoration is complete, the nematodes must be freed of the acid by thorough rinsing in 70 per cent. alcohol when they are ready for further operations. This method has been applied to cestodes, trematodes, arthropods, and their larvae with equally remarkable results.

LITERATURE CITED

Enzyklopädie der Mikroskopischen Technik. 1910. I. 800 pp.

Gilson, G. 1909.—Un nouveau médium solidifiable pour le montage des préparations microscopiques. La Cellule, 23: 428-433.

Lee, A. B. 1913.—The Microtomist's Vade-mecum. 7th. Edition.

NEW HUMAN PARASITES

Stamnosoma armatum Tanabe, 1922.—Certain species of Japanese fresh water fish of the family Cyprinidae are infested with a cercaria which is located in the scales, tail, subcutaneous connective tissue, and viscera. The natural final hosts are stated to be the bird and the cat; dogs, rabbits, rats, and mice also became infested with the adult stage when the cercarial stage was fed to them. The worm becomes fully mature in about a week after feeding. By experiments upon himself the author also proved that the parasite may develop in the human intestine. He states that the egg of this fluke is so similar to that of *Clonorchis sinensis* that in fecal examinations it would seem difficult to distinguish between the two. Tanabe considers that this worm belongs not only to a new species but to a new genus. No description is given in the abstract. (Jour. Kyoto Med. Soc. [Japanese text] 19, No. 3; April, 1922; abstract in Japan Med. World, 2:263-264, Sept. 15, 1922.)

Rhabditis foecalis Watanabe, 1922.—In one of two cases of human infection with Rhabditis in Japan studied by Watanabe, a new species was found. No description is given in the abstract (Japanese Jour. Gastro-enterol., [Japanese text] 21, No. 2; March, 1922; abstract in Japan Med. World, 2:261, Sept. 15, 1922).

Euparyphium jassyense León and Ciurea, 1922.—Flukes found in the stools of a patient of Persian origin, 45 years old, at Jassy, Roumania, proved to belong to a new species. An intermittent and irregular diarrhea in this case was cured by treatment with thymol. The flukes measure about 5.5 to 7.5 mm. in length and resemble somewhat *Echinostomum ilocanum* but with certain differences. The new species differs from other known species of Euparyphium particularly in the location of the testes in the anterior half of the body and in the size of the eggs. It is described in detail and a drawing accompanies the description. (C. R. soc. biol., 87:262-264, 1 fig.; 24 juin, 1922.)

Spirochaeta bilio-hemoglobinuriae Blanchard and Lefrou, 1922.—A spirochete, inoculable into guinea-pigs, was isolated from the blood of a patient suffering from specific blackwater fever (bilious hemoglobinuric fever) in the colonial hospital of Brazzaville. The organisms were discovered in blood drawn on the fourth day of the disease, subjected to the triple centrifugation process of Martin, Leboeuf and Roubaud. They measure 6 to 9μ long by 0.1 to 0.2μ broad and show three or four undulations. Inoculated guinea-pigs almost invariably died from the resultant infection. Spirochetes were recovered from their blood at various times between the first and seventh days after inoculation. An important difference between the symptoms shown by guinea-pigs inoculated with these spirochetes and those inoculated with spirochetes from cases of infective jaundice and yellow fever, is that the former do not become icteric; the cardinal symptom is hemoglobinuria. (C. R. acad. sci., 175:602-604.)

Dibothriocephalus minor Cholodkovsky, 1916.—A member of an expedition sent from Russia to Transbaikalia passed after treatment a tapeworm, which appeared to be of a new species. The strobila was about 10 cm. long, greatest breadth 6 mm. The uterus, apparent 3 cm. from the scolex, had an irregular sac-like outline instead of the characteristic rosette arrangement of the uterus of *D. latus.* The eggs were oval, measuring 70 by 40μ. (J. Russe de Zool., 1:235-237, 1 pl., 6 figs.) [An old item, just coming to light since the war; based on a review in Trop. Dis. Bull.]

BOOK REVIEW

ANIMAL PARASITES AND HUMAN DISEASE. By ASA C. CHANDLER. John Wiley & Sons, Inc., New York, 1922. 572 pp., 254 figs.

The appearance of a new edition of this well-known book is evidence that the original met a distinct demand. In many cases an announced revision is merely an insignificant modification of the original edition. But here the reviewer should record at the outset his satisfaction, which will be generally shared, that this work has really been revised and brought up to date. Additions were made throughout the book sufficient in amount to constitute a significant part of the total. More important still, these additions have been well chosen and satisfactorily incorporated so that the text reads connectedly and the work impresses one as a unit rather than a patch work.

Very naturally the most extended and radical changes are found in the section on Protozoa, where the advance of knowledge during the four years since the appearance of the first edition has been marked. The section on yellow fever has been entirely rewritten and brought into connection with other diseases as warranted by Noguchi's discovery of the causal organism. Some other recently described spirochetes and their relation to the disease have also received due consideration. Among the flagellates insertions have been made to call appropriate attention to the splendid researches of Kofoid and Swezey, as well as other less extended works. The chapter on amoebae has been entirely rewritten and attention given to the extended critical studies of Dobell and others.

Under the Sporozoa the author has introduced considerable material dealing with the organisms of typhus fever, trench fever, spotted fever, and kedani or tsutsugamushi, grouped together as Rickettsia-like organisms. Almost nothing was known of such forms before military necessities compelled attention to these diseases so that we may rightly say the war contributed primarily to our knowledge of these organisms. While the work of Wolbach and his associates on spotted fever and on typhus fever has furnished valuable material utilized in the discussion of these diseases and their causal organisms, unfortunately the most recent of these publications has not received consideration; but this is very likely due to the fact that Chandler's manuscript was closed before the later publications appeared.

Under the Trematodes or flukes, there have been added paragraphs on the treatment and prevention of schistosomiasis, on the life history of Clonorchis and on Goddard's work on Fasciolopsis. However, the very beautiful study of Nakagawa on the life history of the lung fluke and of Kawamura and others on the relations of this parasite to cerebral disease among children have not received attention.

The chapter on Cestodes has suffered less change than any other and naturally because of the lesser amount of work done on these forms. The significant additions in the new edition are a paragraph on the life history of the broad or fish tapeworm of man, and another on the development of *Sparganum mansoni*.

Important items added to the Section on Nematodes include especially the life history of Ascaris, in which, however, entirely inadequate reference is made to the magnificent work of Ransom in this field. Some other significant discoveries concerning Roundworms have not received attention, and there are quite a number of places where minor changes such as were made in the chapter on Protozoa would have greatly improved this section of the book.

The results of scientific work induced by the war appear again conspicuously in the revision of the chapter on lice. The author has wisely enriched his account of these forms by including the splendid work on this group done by Nuttall during the war, and accepts Nuttall's view that these forms are all to be included within a single species having separate races only. But it is not clear why having done so and having used the ordinal name Anoplura in the first edition, Chandler should depart from Nuttall's guidance and replace this name by the less acceptable ordinal designation Siphunculata. The point may appear trivial but is really of fundamental importance in a field like parasitology, involving workers of such widely different training, so that evidently all changes should be avoided unless they are absolutely necessary. Nuttall's work has set the standard and will continue to be followed by medical and Zoological workers in parasitology. The change of a single name is bound to cause confusion and this one is unfortunate also because it does not follow the best of usage in entomology.

Two paragraphs dealing with the development of Dermatobia have been added, one in the section on mosquitoes and the other in connection with the discussion of fly larvae developed under the human skin.

It is a real misfortune that when a revision of this work was undertaken the author should have neglected to replace some of the exceedingly poor figures of the earlier edition that have been critically commented on. In one case (p. 275) where the entire page was rewritten in the new edition, the figure used is little more than a blotch and entirely unworthy of a work of this high character. Many persons will also be inclined to criticize the procedure of the author in omitting to cite at least the major references. There is to be sure a section on the sources of information which is useful even though it has not been brought up to date. Incidentally it would have been a better piece of book printing if this list had not contained so many awkward and unnecessary abbreviations in titles. But even at the best such a list is not adequate for the purposes of the student or just to the work of those investigators on whom the author has leaned for his information. One can but regret that the references are not somewhat more numerous and more directly connected with the text.

NOTES

The Linguatulidae, a group of parasites of marked biological and medical interest, have been subjected to a complete and critical study by L. A. Sambon, whose article in the Journal of Tropical Medicine and Hygiene (25: 188-; June 15, 1922), is of sufficient importance to justify calling it especially to the attention of workers in parasitology.

The Journal of Tropical Medicine and Hygiene for June 15 is a special number commemorating the life and work of the late Sir. Patrick Manson. Two fine photographs and a number of articles discussing his work from various points of view pay well-deserved honor to the memory of this distinguished writer and student of tropical diseases.

The London School of Tropical Medicine has issued two numbers of Collected Papers, embracing 25 important researches on Helminthology as published in various journals by members of its staff. The series, which is a fine testimonial to the activity of this department, will be of real service to workers in this field who can hardly hope to have access ordinarily to all the original sources of publication.

The especial attention of workers in medical zoology is to be called to a monograph by R. B. Seymour Sewell from the laboratory of the Zoological Survey of India, published in the Indian Journal of Medical Research (10, suppl.; June, 1922), in a study covering nearly 400 pages of text and illustrated by 32 full-page plates in color and numerous text figures. The author has given a study of the Cercariae of India that surpasses in extent and thoroughness any study heretofore made of this group in any part of the world. It would be hopeless to attempt a detailed review of so large a work here. Suffice to say that it is sure to be indispensable to all workers in this field.

SPECIFIC NAMES FOR PARASITES

The habit of naming parasitic species after the hosts in which they reside is a frequent one. It is usual to use for the specific name of the parasite the genitive of the genus name of the host, as, *e. g., Giardia muris, Opalina ranarum*. Another convenient form of specific name may be made by using, as a suffix to the genus name of the host, the Greek word for guest (ὁ ξένος, male guest; ἡ ξένη, a female guest). In a dozen or so instances, in naming new species of Opalinidae, this sort of specific name has proven convenient; *e. g., Zelleriella ranaxena* (the guest of *Rana*), *Cepedea bufoxena* (the guest of *Bufo*), *Opalina hylaxena* (the guest of *Hyla*). In case several species of parasites, belonging to one genus are found in one genus of host, it is possible to give to two and often three of the parasites names which refer them to their host; *e. g., Opalina ranarum* (genitive plural of *Rana*), *Opalina ranaxena*, and *Opalina ranarum* form *arvalis* (parasitic in *Rana arvalis*).

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